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ROBUST SUMMARY
OF INFORMATION ON

Substance Group

LUBRICATING OIL BASESTOCKS

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NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)
A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

1.1.1 GENERAL SUBSTANCE INFORMATION

Substance type : Petroleum product
Physical status : Liquid

Remark : The group of base oils consists of products that are derived from both distillates and residues of the vacuum distillation process in petroleum refining.
Base oils consist predominantly of hydrocarbons but may also contain small quantities of sulfur and nitrogen compounds with traces of a number of metals. The oils contain complex hydrocarbons with variable mixtures of paraffins, naphthenes and aromatics with carbon numbers in the range 15 to 50. Hydrocarbon constituents derived from vacuum distillates boil generally in the range 300 to 600 °C, whereas those derived from residual oils may boil up to 800 °C.
Unrefined vacuum distillates contain polycyclic aromatic compounds (PACs) which are removed during any subsequent refining process. The more severe the refining, the lower the PAC content will be of the refined base oil.
Physical chemical data for a range of base oils have been summarized by CONCAWE and these are tabulated in the attached document.
For most of the mammalian toxicology endpoints, information has been used that was derived by the American Petroleum Institute on a wide range of base oils. For simplicity, this robust summary contains detailed information on an API sample of an unrefined distillate (high PAC) and an API sample of a highly refined distillate (low PAC). If data was available on other samples, it has either been summarized in tabular form in the relevant sections of this summary or discussed in detail when appropriate.
The API sample of highly refined base oil for which data have been selected is one with a low average molecular weight since this is likely to represent the worst case from a toxicological perspective.
The physico-chemical characteristics of the two samples are as follows:

	Method	Unrefined oil	Highly refined oil
API sample No.		84-01	83-12
CAS No.		64741-50-0	64742-53-6
API Gravity @60°	D287	31.9	25.9
Density @15°C	D287	0.8651	0.8981
Molecular wt. (gm/mol)	D2224	300	260
Refractive index (RI units @20 °C)		1.4815	1.4910
Total Sulfur (wt. %)	D3120	0.38	0.04
Total Nitrogen (ppm/wt)	Chemil	210	38
Total oxygen (wt.%)	NAA	0.038	0.077
Total Chloride (ppm/wt)	coulom	11	2
Viscosity (cSt @ 40°C)	D445	14.07	0.44
Viscosity (cSt @ 100°C)	D445	2.79	2.14
Pour point (°F)	D93	+60	<-20
Carbon residue (wt. %)	D524	0.15	0.14
Distillation	D1160		
IBP (°F)		595	450
FBP (°F)		810	785
Hydrocarbon type analysis			
Nonaromatics (wt. %)	D2549	79.1	67.3
Aromatics (wt. %)	D2549	20.9	31.9
TOTAL		100	100

Some oils are destined for food use or pharmaceutical applications and for these the refining process that they undergo is particularly severe to ensure that aromatic materials have been removed and that the resulting oil is colorless. Such oils are known as white oils. Unlike the other base oils in which oral intake is unintentional, the white oils are intended for uses in which an oral intake is likely. For these materials, oral studies are available and, where appropriate, are included in this Robust Summary.
Several individual companies have generated data on environmental effects and ecotoxicity. The relevant CAS descriptions of the materials that have been tested are included in the relevant sections of this robust summary.

Attached document : Attachment 1: Physico-chemical data

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1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit : TLV (US)
Limit value : 5 mg/m^3
Short term exposure limit value
Limit value : 10 mg/m^3

Remark : A TWA TLV of 0.005 mg/m^3 is proposed for the sum total of 15 polynuclear aromatic hydrocarbons (PAHs) listed as carcinogens by the U.S. National Toxicology Program (NTP).

(1)

1.13 REVIEWS

Memo : IARC reviewed the carcinogenicity information on lubricating base oils and the outcome of their review was published in a Monograph (IARC 1984).

(101)

Memo : Bingham reviewed the literature for information on the carcinogenic potential of petroleum hydrocarbons. This review contained information on base oils.

(23)

Memo : CONCAWE demonstrated that it was possible to distinguish between carcinogenic and non-carcinogenic base oils on the basis of the level of DMSO extractables.

Remark : The DMSO method was adopted subsequently in the EU to distinguish between carcinogenic and non-carcinogenic oils for classification and labeling purposes.

(80) (86)

Memo : The EU Scientific Committee for Food (SCF) and the WHO Joint Expert Committee on Food Additives (JECFA) have reviewed the available data on the toxicology of mineral hydrocarbons for food uses.

(102) (112)

Memo : The WHO published an Environmental Health Criteria document which included summarized information on lubricating base oil stocks

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2.1 MELTING POINT

Sublimation :
Method : ASTM D97
GLP : No data
Test substance : Lubricating Base Oils; distillate oils, residual oils, and white oils

Remark : By definition, melting point is the temperature at which a solid becomes a liquid at normal atmospheric pressure. For complex mixtures like petroleum products, melting point may be characterized by a range of temperatures reflecting the melting points of the individual components. To better describe phase or flow characteristics of petroleum products, the pour point is routinely used. The pour point is the lowest temperature at which movement of the test specimen is observed under prescribed conditions of the test (ASTM 2002). In addition, the pour point methodology defines a "no-flow" point, defined as the temperature of the test specimen at which a wax crystal structure or viscosity increase, or both, impedes movement of the surface of the test specimen under the conditions of the test (ASTM 2002). Because not all petroleum products contain wax in their composition, the pour point determination encompasses either change in physical state (i.e., crystal formation) and/or viscosity property.

Result : See following Table and Remarks Section

Oil type	Pour Point, °C
Distillate Oils	
Unrefined/Mildly Refined	
Distillate, light paraffinic (CAS No. 64741-50-0)	15.5
Highly/Severely Refined	
Solvent de-waxed, light paraffinic (CAS No. 64742-56-9)	-18
Solvent de-waxed, heavy paraffinic (CAS No. 64742-65-0)	-12
Hydrotreated, light paraffinic (CAS No. 64742-55-8)	-18
Hydrotreated, heavy paraffinic (CAS No. 64742-54-7)	-9
Hydrotreated, light naphthenic (CAS No. 64742-53-6)	-60
Hydrotreated, heavy naphthenic (CAS No. 64742-52-5)	-24
White mineral oil (CAS No. 8042-47-5)	-15
Residual Oils	
Solvent de-waxed (CAS No. 64742-62-7)	-6

Reliability : (2) valid with restrictions
Results of standard method testing was reported in a reliable review dossier.

(18) (19) (81)

2.2 BOILING POINT

Method : American Society for Testing and Materials (ASTM) Method D1160, Standard Test Method for Distillation of Petroleum Products at Reduced Pressure.

GLP : No

Test substance : CAS Nos. 64741-50-0; 64742-53-6; 64742-52-5

Remark : The substances covered in lubricating base oils are complex and variable mixtures of paraffinic, naphthenic (cycloparaffins), and aromatic hydrocarbon compounds having carbon numbers ranging from approximately 15 to 50. Because they are mixtures, lubricating base oils do not have a single numerical value for boiling point, but rather a boiling range that reflects the individual components. Lubricating base oils are produced from vacuum distillation of the residue obtained after the atmospheric distillation of crude oil. The vacuum distillates and the vacuum residues together form the general group of unrefined or mildly refined base oil. Additional treatments or refinements such as solvent extraction, dewaxing, and hydrogenation, are employed to produce oils with desirable properties.

Result :

Lube Oil Streams	Distillation Range, °F (°C)	Ref.
Distillates Streams		
unrefined/mildly refined (C_{15} - C_{30} range) CAS No. 64741-50-0	595 - 810 (313 - 432)	API 1987
highly/severely refined (C_{15} - C_{30} range) CAS No. 64742-53-6	450 - 785 (232 - 418)	API 1987
highly/serverely refined (C_{20} - C_{50} range) CAS No. 64742-52-5	640 - 1120 (338 - 604)	API 1987
Residual Streams		
vacuum residues	up to 1472 (800)	CONCAWE 1997

Reliability : (2) valid with restrictions

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2.4 VAPOUR PRESSURE

Method	:	Directive 84/449/EEC, A.4 "Vapour pressure"
Year	:	1991
GLP	:	Yes
Test substance	:	CAS No. 64742-65-0, Distillates (petroleum), solvent-dewaxed, paraffinic
Result	:	Three runs on the sample were conducted. There was initially substantial reduction (equivalent to 3°C temperature change) of estimated VP on prolonged pumping after Run 1 but this was reduced to the equivalent of 0.65°C change between Runs 2 and 3. The latter runs provided values at room temperature of 1.882 and 1.563 x 10^{-4} Pascals, yielding a mean value of Vp(298.15K) = 1.723 x 10^{-4} Pascals. The condensation rates onto the pan observed in Run 3 increased with temperature more rapidly than the mass difference indicating an increasing efficiency of condensation and thus precluding the use of the condensation data to produce a satisfactory VP relation. The final values of rate of condensation were however equivalent in pressure regime to the mass differences assuming a rough equality between the numerical magnitudes of temperature and molar mass.
Test condition	:	The vapor pressure (VP) was determined using a VP balance based on a CI Electronics micro-balance with a sensitivity of approximately 0.1 mg. Sample temperature was controlled electronically (±1°C) over the range from ambient to 250°C. Mass readings and temperature were recorded directly onto a 2-channel chart recorder. The VP balance was designed such that on opening the slide across the orifice in the temperature controlled evaporation furnace, the escaping vapor jet was directed at the scale pan. VP was determined directly from the pressure on the scale pan by measuring the difference of mass readings when the slide across the orifice was open and closed. When condensation occurred onto the pan the VP can be calculated from the condensation rate if the molar mass is known. VP of the sample was measured at several temperatures to yield VP curves for subsequent extrapolation to give 298.15K values. Slope and intercept of VP curve were estimated by an unweighted least squares statistical treatment of the data and errors are ± standard deviation of the respective quantity. Maximum and minimum values of VP at 298.15K were calculated directly from the VP relationship using the ranges of errors in slope and intercept respectively. The quoted errors in VP at 298.15K were then calculated directly by extrapolation from these values.
Reliability	:	(2) valid with restrictions The reported measurement was below the method limit of sensitivity.

(100)

Method	:	Calculated: EPIWIN, MPBPWIN V1.40 computer model (U.S. EPA, 2000)
Year	:	2000
GLP	:	No
Test substance	:	Distillate unrefined, refined, highly refined and residual lubricant base oils
Remark	:	MPBPWIN V1.40 was used to calculate the vapor pressures of representative hydrocarbon constituents in lubricant base oils. Because lube oils may cover C_{15} to C_{50} and C_{20} to C_{50} hydrocarbon mixtures, vapor pressure estimates are provided for common structures covering those ranges.

The estimates given below are values for single structures. Since the total vapor pressure of mixtures is a function of its vapor pressure as a pure substance multiplied by its mole fraction in the mixture (Raoult's law), complex mixtures such as lube oils would be expected to have exceedingly low total vapor pressures.

Result :

	Vapor Pressure, Pa		
Structure	**C15**	**C20**	**C50**
n-paraffin	4.57E-01	6.16E-04	1.97E-07
iso paraffin	5.81E+00	1.88E-01	8.44E-13
1-ring naphthene	3.31E-01	2.17E-02	2.12E-13
2-ring naphthene	2.67E+00	2.33E-02	2.17E-13
3-ring naphthene	2.33E+00	2.11E-02	2.23E-13
1-ring aromatic	7.61E-01	3.17E-03	1.99E-14
2-ring aromatic	5.33E-02	7.26E-04	3.12E-15
3-ring aromatic	8.89E-03	1.05E-04	4.87E-16

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.

2.5 PARTITION COEFFICIENT

Method : Calculated: EPIWIN©, KOWWIN V1.66 computer model (U.S. EPA, 2000)
Year : 2000
GLP : No
Test substance : Distillate unrefined, highly refined and residual lubricant base oils.

Remark : Unrefined and highly refined base oils consist principally of hydrocarbons covering the carbon number range C15 to C50. They consist of the following generic types of hydrocarbon: straight and branched chain alkanes, naphthenes i.e. mono, di-, tri-, tetra- etc, cycloalkanes, aromatic hydrocarbons including alkylbenzenes and 2 to 7 fused ring compounds. From the carbon number range of the hydrocarbon constituents, it may be deduced that the log Kow range of these substances will extend from 4.5 to over 6.

Result :

	Partition Coefficient, Log Kow		
Structure	**C15**	**C20**	**C50**
n-paraffin	7.7	10	25
iso paraffin	7.6	10	25
1-ring naphthene	7.5	10	25
2-ring naphthene	6.6	9.0	24
3-ring naphthene	5.6	8.1	23
1-ring aromatic	7.1	8.9	24
2-ring aromatic	5.7	8.1	23
3-ring aromatic	5.2	7.4	22

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Method : EPIWIN©, WSKOWWIN V1.40 computer model (U.S. EPA, 2000)
Year : 2000
GLP : No
Test substance : Distillate unrefined, highly refined and residual lubricant base oils.

Remark : It is predicted from calculations of solubility behavior that paraffinic, naphthenic and aromatic hydrocarbons in the range of C15 toC50 will have extremely low water solubilties, due to the hydrophobic behavior of these high molecular weight hydrocarbons. Estimated water solubility values for representative C15, C20, and C50 hydrocarbons for paraffinic, naphthenic and aromatic class ranges from 0.6 mg/l to <0.001 mg/l.

Result :

	Water Solubility, mg/l		
Structure	**C15**	**C20**	**C50**
n-paraffin	7.6E-05	1.9E-03	5.3E-21
iso paraffin	3.0E-03	1.3E-05	7.1E-21
1-ring naphthene	4.0E-03	1.4E-05	7.9E-21
2-ring naphthene	3.0E-02	9.1E-05	5.2E-20
3-ring naphthene	1.8E-01	6.0E-04	3.4E-19
1-ring aromatic	3.5E-02	4.1E-04	2.4E-19
2-ring aromatic	6.3E-01	2.1E-03	1.2E-18
3-ring aromatic	2.8E-01	8.3E-04	4.9E-19

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.

3.1.1 PHOTODEGRADATION

Deg. product :
Method : Calculated): Calculations by EPIWIN V3.10; AOPWIN V1.90.
Year : 2000
GLP : No
Test substance : CAS No.: Various; Unrefined and acid treated base oils.

Remark : AOPWIN V1.90 calculates atmospheric oxidation half lives of hydrocarbons in contact with hydroxyl radicals in the troposphere, under the influence of sunlight. Atmospheric oxidation rates were calculated for a range of molecular structures (paraffinic, naphthenic and aromatic hydrocarbons) and different molecular weights (i.e., C_{15}, C_{20} and C_{50}) for hydrocarbon components in lubricating base oils.
Although the low vapor pressures of these base oils indicate that volatilization will not be a very significant fate process, oxidation half-lives indicate this may be a moderate removal process if these substances were introduced to the atmosphere by adsorption to particulate matter via atmospheric emissions. The half-lives for degradation of these hydrocarbons by reaction with hydroxyl radicals, in the troposphere, under the influence of sunlight, will all be less than one day, by extrapolation from the data quoted by Atkinson (1990).

In general, most products in the base oil category do not contain component molecules that will undergo direct photolysis. Saturated hydrocarbons (paraffins and naphthenics), and single ring aromatics, which constitute the majority of these components, do not absorb appreciable light energy above 290 nm. Therefore, direct photolysis will not contribute to a measurable degradative removal of chemical components in this category from the environment.

Result : Indirect photolysis at 25 °C
Concentration of sensitizer: 1.50×10^{6} OH radicals/cm^3
Rate constant: 18.1757×10^{-12} cm^3/mol-sec
Half-life: See following table

Atmospheric Oxidation Half-Life, days

Structure	C_{15}	C_{20}	C_{50}
n-paraffin	0.6	0.4	0.2
iso-paraffin	0.6	0.4	0.2
1-ring naphthene	0.5	0.4	0.2
2-ring naphthene	0.4	0.3	0.1
3-ring naphthene	0.4	0.3	0.1
1-ring aromatic	0.7	0.5	0.2
2-ring aromatic	0.2	0.2	0.1
3-ring aromatic	0.3	0.3	0.1

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.
(21) (82) (124)

3.1.2 STABILITY IN WATER

GLP : No

Result :
Measured value: N/A
Degradation %: N/A
Half-life: N/A
Breakdown products: N/A

Conclusion : Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkylhalides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters. The chemical components that comprise the base oil category are hydrocarbons, which are not included in these chemical groups, and they are not subject to hydrolysis reactions with water.

Reliability : (1) valid without restriction

(99)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : Mathematical computer model

Media : Soil, air, water, suspended sediment and sediment for C15 hydrocarbon structures

Method : Calculations by EQC V2.11

Year : 1999

Remark : Model based on chemical fugacity. Multimedia distribution was calculated for C15, C20 and C50 hydrocarbons. Some low molecular weight hydrocarbons will partition to air, while larger molecular weight components are expected to exhibit greater partitioning behavior to terrestrial media. Mobility in the aquatic and atmospheric environment is low due to low water solubility and low vapor pressure. These components will partition rapidly to the terrestrial compartment, where the main fate process is expected to be moderate to slow biodegradation of base oil components in soil and sediment.
A summary of the EQC modeling of the distribution and transport between environmental compartments for selected hydrocarbon compounds in lubricant base oils is presented in the attached table and graph. The compounds selected for modeling represent various C15, C20 and C50 compounds in base oils (e.g., linear and branched paraffins, naphthenes and aromatic hydrocarbons). The default model input properties used in EQC are as follows:

	Volumes (m^3)	Densities (kg/m^3)	Organic Carbon (g/g)	Lipid (g/g)
Air	$1x10^4$	1.185		
Water	$2x10^{11}$	1000		
Soil	$8.9x10^9$	2400	0.02	
Sediment	$1x10^8$	2400	0.04	
Susp. Sed.	$1x10^6$	1500	0.2	
Fish	$2x10^5$	1000		0.05
Aerosol	$2x10^3$	2000		

Result : The following table summarizes the modeling results for the different C15, C20 and C50 hydrocarbon structures covering the range of molecular

weights in lubricating base oil mixtures.

Percent Distribution in Environmental Media

	Air	Water	Soil	Sediment	Suspended Sediment	Biota
n-paraffin						
C15	13	<0.1	85	2	<0.1	<0.1
C20	<0.1	<0.1	98	2	<0.1	<0.1
C50	<0.1	<0.1	98	2	<0.1	<0.1
iso-paraffin						
C15	68	<0.1	31	0.7	<0.1	<0.1
C20	9	<0.1	89	2	<0.1	<0.1
C50	<0.1	<0.1	98	2	<0.1	<0.1
1-ring naphthene						
C15	0.4	<0.1	97	2	<0.1	<0.1
C20	1	<0.1	97	2	<0.1	<0.1
C50	<0.1	<0.1	98	2	<0.1	<0.1
2-ring naphthene						
C15	51	<0.1	48	1	<0.1	<0.1
C20	2	<0.1	96	2	<0.1	<0.1
C50	<0.1	<0.1	98	2	<0.1	<0.1
3-ring naphthene						
C15	58	0.1	41	1	<0.1	<0.1
C20	2	<0.1	96	2	<0.1	<0.1
C50	<0.1	<0.1	98	2	<0.1	<0.1
1-ring aromatic						
C15	34	0.2	65	1	<0.1	<0.1
C20	<0.1	<0.1	98	2	<0.1	<0.1
C50	<0.1	<0.1	98	2	<0.1	<0.1
2-ring aromatic						
C15	0.7	0.2	97	2	<0.1	<0.1
C20	<0.1	<0.1	98	2	<0.1	<0.1
C50	<0.1	<0.1	98	2	<0.1	<0.1
3-ring aromatic						
C15	2	1	95	2	<0.1	<0.1
C20	<0.1	<0.1	98	2	<0.1	<0.1
C50	<0.1	<0.1	98	2	<0.1	<0.1

Conclusion : This complex petroleum mixture is expected to partition primarily to soil and/or sediment.

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.
(82) (122)

3.5 BIODEGRADATION

Type : Aerobic
Inoculum : Microorganisms were obtained from Canterbury Sewage Works (UK) and prepared according to the prescribed methods for this test.
Contact time : 28 day(s)
Method : Directive 84/449/EEC, C.5 "Biotic degradation - modified Sturm test"
Year : 1986
GLP : Yes
Test substance : CAS No. 64742-65-0; Distillates (petroleum), solvent-dewaxed heavy paraffinic

Result : The test substance was partially degraded to 20-26% of the theoretical CO_2 in 28 days. Degradation commenced after a lag period of 2 days. Biodegradation curve showed that degradation had virtually stopped by day 28. Test substance was therefore inherently biodegradable since it achieved >20% biodegradability based upon CO_2 evolution.

Sample	% Degradation (day 28)	Mean % Degraded
Test substance	26, 20	23
Na Benzoate	86, 92	89

Test condition : The test substance was added to test medium from a stock solution containing 2.4 g/l emulsified in Dobane PT sulphonate (2 mg/l), a non-biodegradable detergent. The final test concentration of the base oil was 20 mg/l. The test medium was dispensed into Sturm vessels, inoculated and aerated with 60 ml/min of CO_2-free air and incubated at 20 ± 1°C. Biodegradation was determined on days 1, 2, 5, 9, 14, 20, and 28 by titrating the total CO_2 released. The medium was acidified on day 27 to release the total CO_2 by day 28. Test substance, control blank, and sodium benzoate control (20 mg/l) were tested in duplicates. The empirical formula used was C_nH_{2n+1} which yielded a theoretical CO_2 evolution of 3.14 g CO_2 per g of test substance.
Reliability : (2) valid with restrictions
The study report lacked an extensive description of experimental procedures but instead referenced procedures detailed in a laboratory SOP.

(116)

Type : Aerobic
Inoculum : Activated sludge, domestic
Contact time : 28 day(s)
Method : OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Year : 1995
GLP : Yes
Test substance : CAS No. 64742-54-7; Distillates (petroleum), hydrotreated heavy paraffinic

Result : By day 28, 31% degradation of the test material was observed and indicated that the test material was inherently biodegradable. By day 5, >60% biodegradation of positive control was observed, which meets the guideline requirement. No excursions from the protocol were noted. Biodegradation was based on net oxygen consumption and the theoretical oxygen demand of the test material as calculated using results of an elemental analysis of the test material.

Sample	% Degradation* (day 28)	Mean % Degradation (day 28)
HHP	32.93, 27.2,33.27	31.13
Na Benzoate	82.04; 72.88	77.46

* replicate data

Test condition : Fresh activated sludge was obtained one day prior to test initiation, and homogenized in a blender for two minutes. After allowing the sample to settle for approximately 30 minutes, the homogenated supernatant was decanted, avoiding carry-over of solids. Microbial activity of an aliquot of the filtered supernatant was $1E^6$ CFU/ml which was determined using microbial agar dip slides. Activated sludge supernatant was added to the test medium at 10 ml/l and the inoculated medium was continuously aerated with CO_2-free air until the next day when the test systems were prepared.
Test medium consisted of glass distilled water and mineral salts (phosphate buffer, ferric chloride, magnesium sulfate, calcium chloride). Test vessels were 1 Liter glass flasks located in a water bath and electronically monitored for oxygen consumption. Test material was tested in triplicate, controls and blanks were tested in duplicate. Test material (hydrotreated heavy paraffinic petroleum distillates, HHP) concentration was approximately 44 mg/l, equivalent to a theoretical oxygen demand (ThOD) of 148 mg/l. Test material was weighed onto a Gelman type A/E 13 mm glass fiber filter which was then added to each respirometer flask. Sodium benzoate (positive control) concentration was 53.54 mg/l, and was added using an aliquot of a stock solution. Test temperature was 22 ± 1°C. All test vessels were stirred constantly for 28 days using magnetic stir bars and plates.

Reliability : (1) valid without restriction

(95)

Type : Aerobic
Inoculum : Activated sludge, domestic
Contact time : 28 day(s)
Method : OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO2 evolution)"
Year : 1990
GLP : Yes
Test substance : CAS No. 64741-89-5; distillates (petroleum), solvent-refined, light paraffinic

Result : By day 28, the 10 and 20 mg C/L test flasks showed

Day	% Degradation Reference	% Degradation 10 ppm Test Sub.	% Degradation 20 ppm Test Sub.
10	31	0	1
21	89	25	12
28	89	29	22

The test material was not readily biodegradable. Within a period of 28 days, 22 and 29% degradation was observed. The pass limit for this test is 60% within 28 days.
The reference test substance was degraded to 89% by day 28. The pH of the test cultures (10 mg/l and 20 mg/l) and controls (sodium benzoate standard and negative control) measured on Day 27 were 4.8, 4.8, 4.9, and 5.2, respectively.

Test condition : The test material entered the experimental containers through direct

dispersion in water. Activated sludge bacteria from the Severn Trent Plc sewage treatment plant in Belper, Derbyshire was used as the inoculum. The sample sludge was homogenized in a mixer for 10 minutes prior to a solid settling phase and a subsequent filtering of the supernatant for use. The experimental containers had an inoculum concentration of 1%. The exposures lasted for a period of 28 days. The experimental containers were 5 liter glass culture vessels, containing 3 liters of a mixture of nutrient medium, test material, and inoculum. Test conditions were run in darkness at a constant temperature of 21°C. Nutrient medium was prepared according to the OECD guideline recipe using tap water purified by ion exchange and reverse osmosis.
A series of both two controls and two test material concentrations were run. The controls consisted of a group with just the culture medium and the inoculum and a group with culture medium, inoculum, and 20 mg/l Sodium benzoate (C_6H_5 * COONa). The two test concentrations of test material were 10 and 20 mg/l.
All culture vessels were sealed and aerated with CO_2 free air at a rate of about 2 bubbles per second. Additionally, the solution was continuously stirred by magnetic stirrers.
Samples were taken from the first CO_2 absorber vessel on Days 0, 1, 2, 3, 6, 8, 10, 14, 16, 21, 23, 27, and 28. Samples were taken from the second absorber vessel on Days 0 and 28. The absorbers were made up of 500 ml Dreschel bottles filled with 350 ml of 0.05M NaOH. The solution was prepared using purified, degassed water. On day 27, the pH of each vessel was measured and 1 ml of concentrated HCl was added to drive off inorganic carbonate. CO_2 production (as inorganic carbon) was measured by an Ionics 555 TOC Analyzer in triplicate.

Reliability : (2) valid with restrictions
The study was performed following the 1981 guidelines for OECD 301B. (36)

Type : Aerobic
Inoculum : Activated sludge, domestic
Contact time : 21 day(s)
Method : CEC Method L-33-T-82 using test medium from ISO Standard 7827 and OECD 301A and 301E
Year : 1991
GLP : Yes
Test substance : CAS No. 64741-89-5; distillates (petroleum), solvent-refined, light paraffinic

Result : By day 21, biodegradation of the test substance was 63%, 65%, and 61% in the individual flasks. The mean biodegradation was 63%.

% Biodegradation

	Reference Material			Test Substance		
Day	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3
21	27	29	30	63	65	61
Mean:		29			63	

Biodegradation of the reference material was 27%, 29%, and 30% in the individual flasks, and the mean biodegradation was 29%.
There were no apparent deviations from the given method.

Test condition : Settled activated sludge acquired from Buckland Sewage Treatment Works, Milber, Newton Abbot, Devon, was utilized as the inoculum. The inoculum was normally between 10^5 and 10^7 Colony Forming Units (CFU)/ml. Bacteria were enumerated by Dip Slide (Oxoid, TTC Red Spot) and incubated at 25 ±1°C until sufficient colonies were visible to enable counting.
The inoculum was used in the experiment at a rate of 1 ml per flask.

The test medium was prepared following the formula specified in ISO Standard 7827. Mother solutions of the test substance and reference oil were prepared by adding 150 g of test or reference substance to 1 liter of A113 (1,1,2-trichlorotrifluoroethane). The negative control reference substance was white oil, R.L. 110 (Brixham test substance #T071). The test design consisted of 5 test flasks containing 150 ml of test medium, 1 ml inoculum, and 50 ml of test substance mother solution; 5 reference flasks containing 150 ml of test medium, 1 ml inoculum, and 50 ml of reference substance mother solution; 2 blank flasks containing 150 ml of test medium and 1 ml inoculum; and 1 poisoned flask prepared identical as the test flasks but contained 1 ml of $HgCl_2$. Incubation flasks were 500-ml conical flasks fitted with foam plugs.
On day 0 of the test, two blank flasks, two test flasks, and two reference flasks were sacrificed for analysis of residual oil content by infrared spectrophotometry (see analysis procedure below). The remaining flasks were placed on an orbital incubator and maintained at 25 ± 1°C for 21 days. On day 21, the contents of all flasks were analyzed for residual oil content.
Analysis Procedure:
Residual oil content (%) in each flask was analyzed using a method suitable for the determination of hydrocarbon lubricants in water samples. Lubricants were extracted from water using 1,1,2 trichlorotrifluoroethane and were analyzed using infrared spectrophotometry. The samples were quantified against known standards of the lubricant using the maximum absorption of the CH_3-CH_2 band at 2930 ± 10 cm^{-1}.
Percent test substance degraded was calculated as

$$\frac{\%\text{ (ROC) poisoned flask} - \%\text{ ROC test flask}}{\%\text{ROC poisoned flask}} \times 100$$

Reliability : (2) valid with restrictions
The CEC method is not a test of ready or inherent biodegradability, nor do the test results provide a reliable measure of the extent of ultimate biodegradability, or mineralization. These test results can only indicate primary biodegradation, i.e., some loss of oil based on concentration analysis of the parent base oil over the course of the study.

(59)

Type : Aerobic
Test substance : Various base oils

Result : 28 biodegradability studies have been reported for base oils.
In the preceding paragraphs a full study description is Based on the results of ultimate biodegradability tests using modified Sturm and manometric respirometry testing these base oils are expected to be, for the most part, inherently biodegradable.
Results of primary biodegradability testing using the CEC test method indicate that transformation of parent base oil due to biological activity occurs to a varying extent, ranging from 13% to 79% loss of original concentrations of tested base oils.

Summarized data for all studies (including those described in the preceding paras) are tabulated below

Method*	Biodeg. (%)	Ref.
Distillates, solvent-refined heavy paraffinic (64741-88-4)		
OECD 301B**	22, 11	BP AT301/036
OECD 301B	15, 12	BP AT301/030
OECD 301B	8, 8	BP AT301/032
OECD 301B	3, 11	BP AT301/035
OECD 301B	12, 11	BP 301/031
OECD 301B	9, 8	BP AT301/034
CEC L-33-T-8272		BP BL3821/B
CEC L-33-T-8271		BP BL3822/B
CEC L-33-T-8253		BP BL3823/B
CEC L-33-T-8279		BP BL3820/B
CEC L-33-T-8264		BP BL3826/B
CEC L-33-T-8251		BP BL3825/B
Distillates, solvent-refined light paraffinic (64741-89-5)		
OECD 301B	29, 22	BP AT301/064
OECD 301B	17, 17	BP AT301/029
CEC L-33-T-8263		BP BL3975/B
CEC L-33-T-8275		BP BL3819/B
Solvent de-asphalted Bright stock (64741-95-3)		
OECD 301B	11, 4	BP AT301/038
CEC L-33-T-82	17	BP BL3971/B
Distillates, hydrotreated or solvent refined light naphthenic (64741-97-5)		
84\449\EEC, C5	1.5	Shell SBGR.87.259
Solvent-refined residual oil (64742-01-4)		
OECD 301B	4, 2	No Ref
OECD 301B	5, 5	BP AT301/037
CEC L-33-T-82	45	BP BL3824/B
CEC L-33-T-82	13	BP BL3970/B
Distillates, hydrotreated or solvent refined light naphthenic (64742-53-6)		
OECD 301F	42	EBSI 107194A
Distillates, hydrotreated heavy paraffinic (64742-54-7)		
OECD 301F	31	EBSI 198194A
Distillates, solvent dewaxed light paraffinic (64742-56-9)		
OECD 301F	50	EBSI107094A
Distillate, solvent-dewaxed heavy paraffinic (64742-65-0)		
84\449\EEC, C5	23	Shell SBGR.86.137
OECD 301F	38	EBSI 123694A
White oil, (8042-47-5)		
OECD 301B*** -,	24	cited in CONCAWE 97/108
CEC L-33-T-820		cited in CONCAWE 97/108

* Methods used are:
OECD 301B Ready, Sturm test
OECD 301F Ready, Manometric method
CEC L-33-T-82 CEC Test
84\449\EEC, C5 Ready, Sturm Test

** For method OECD 301B the two values given for biodegradation are for test material concentrations of 10

*** Value only available for 20 ppm concentration

(29) (30) (31) (32) (33) (34) (35) (36) (37) (48) (53) (54) (55) (56) (57) (58) (59) (60) (61) (62) (63) (91) (92) (93) (94) (116) (117)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	Semistatic
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Limit test	:	Yes
Analytical monitoring	:	Yes
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	1990
GLP	:	Yes
Test substance	:	CAS No. 64741-89-5; distillates (petroleum), solvent-refined, light paraffinic
Result	:	No mortality at 96 hours in the 0 and 1000 mg/l groups. 96 hrs-LL_0 = 1000 mg/l, based on nominal loading rates. Only one concentration was tested in the limit test. The report states that water samples were taken at 0, 24, and 96 hours for analytical verification of test concentrations, but results of any analyses were not reported.
Test condition	:	Daily renewal of the test media ensured that test material levels were maintained at the exposure concentrations. The test media was introduced into the exposure vessels through direct dispersion in water. Shielded propeller-stirrers were utilized to aid in the dispersion of the test material. Observations indicated that the test material was well dispersed throughout the experiment. 20 ml water samples were drawn from the exposure vessels via a glass syringe and delivered to a storage vessel. The syringe was then rinsed with 20 ml of 1,1,2-trichlorotrifluoroethane. Subsequently, the rinse was mixed with the sample prior to storage. Water samples were collected at 0, 24, and 96 hours into the experiment. Samples were stored at 4°C in glass containers until BP International Limited analyzed them. Exposure vessels were glass aquaria equipped with shielded propeller-stirrers containing 20 liters of test media. The stirrers rotated at 2000 rpm. 10 fish were housed in each vessel and 20 fish were exposed at the experimental concentration. The experimental groups included a control and a group exposed to a concentration of 1000 mg/l. The exposure was conducted under a 16 hour/8 hour, light/dark photoperiod. The rainbow trout were supplied by Trafalgar Nurseries, Downton, Salisbury, U.K. The mean length and mean weight (sd) of the experimental fish were 4.8 cm (0.4 cm) and 1.33 g (0.49 g), respectively. Fish were fed commercial trout pellets on a daily basis. Feeding was discontinued 24 hours prior to the initial exposure. The fish were laboratory acclimated for 4 days prior to a one week test condition acclimation. Biomass loading in the test chambers was 0.67 g/l. Test water was tap water, dechlorinated through the addition of sodium thiosulfate. Exposures occurred at 14°C, a hardness of 50 mg/l as $CaCO_3$ and the D.O. level never dropped below 10.0 mgO_2/l. The pH of the control groups ranged from 7.6-7.7.
Reliability	:	(2) valid with restrictions Only one concentration of the test substance was tested. Results of chemical analyses of test substance concentrations were not reported.

(46)

Type	:	Semistatic
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	Yes
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	1990
GLP	:	Yes
Test substance	:	Solvent-refined residual oil, CAS No. 64742-01-4
Result	:	No mortality at 96 hours in the 0 and 1000 mg/l groups. 96 hrs-LL_0 = 1000 mg/l, based on nominal loading rates. Only one concentration was tested in the limit test. The report states that water samples were taken at 0, 24, and 96 hours for analytical verification of test concentrations, but results of any analyses were not reported.
Test condition	:	Daily renewal of the test media ensured that test material levels were maintained at the exposure concentrations. The test media was introduced into the exposure vessels through direct dispersion in water. Shielded propeller-stirrers were utilized to aid in the dispersion of the test material. Observations indicated that the test material was well dispersed at the beginning of the experiment. After 24 hours the test material was observed adhering to the glassware and propeller shields, as well as forming a floating scum on the water surface. This was also observed at each additional 24 hour renewal period. 20 ml water samples were drawn from the exposure vessels via a glass syringe and delivered to a storage vessel. The syringe was then rinsed with 20 ml of 1,1,2-trichlorotrifluoroethane. Subsequently, the rinse was mixed with the sample prior to storage. Water samples were collected at 0, 24, and 96 hours into the experiment. Samples were stored at 4oC in glass containers until BP International Limited analyzed them. Exposure vessels were glass aquaria equipped with shielded propeller-stirrers containing 20 liters of test media. The stirrers rotated at 2000 rpm. 10 fish were housed in each vessel and 20 fish were exposed at the experimental concentration. The experimental groups included a control and a group exposed to a concentration of 1000 mg/l. The exposure was conducted under a 16 hour/8 hour, light/dark photoperiod. The rainbow trout were supplied by Trafalgar Nurseries, Downton, Salisbury, U.K. The mean length and mean weight (sd) of the experimental fish were 4.8 cm (0.4 cm) and 1.33 g (0.49 g), respectively. Fish were fed commercial trout pellets on a daily basis. Feeding was discontinued 24 hours prior to the initial exposure. The fish were laboratory acclimated for 4 days prior to a one week test condition acclimation. Test water was tap water, dechlorinated through the addition of sodium thiosulphate. Exposures occurred at 14°C, a hardness of 50 mg/l as $CaCO_3$ and the D.O. level never dropped below 10.0 mgO_2/l. The pH of the control groups ranged from 7.6-7.8.
Reliability	:	(2) valid with restrictions Only one concentration of the test substance was tested. Results of chemical analyses of test substance concentrations were not reported.

(28)

Type	:	Semistatic
Species	:	Oncorhynchus mykiss (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	Yes
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	1994
GLP	:	Yes
Test substance	:	Distillate aromatic extract (CAS 64742-04-7)

Remark : A 24-hour WAF mixing period was selected based upon a mixing trial using a similar product. No substantial differences in the total organic carbon content in the aqueous phase were seen between 24 and 48-hour mixing periods.

Result : There was no mortality or other adverse reactions to the exposures during or after 96 h in the control and 1000 mg/l test solutions. Inspection of the data revealed the following:

Highest test concentration resulting in 0% mortality: 1000 mg/l WAF

Lowest test concentration resulting in 100% mortality: >1000 mg/l WAF

No Observed Effect Level (NOEL): 1000 mg/l WAF

Total organic carbon analyses results (mg/l):

Treatment Group	0-h	24 h	72 h	96 h
Control	6.020	2.813	3.760	4.011
1000 mg/l Rep 1	5.460	3.211	4.457	3.859
1000 mg/l Rep 2	4.952	2.620	3.849	3.779

Total organic carbon measurements made in the exposure solutions during the test were variable. The authors claim that the carbon analyses do not provide definitive evidence of stability of the test preparations.

Test condition : A semi-static toxicity test was conducted with daily renewal of test solutions. Test solutions were prepared as water accommodated fractions (WAF). Nominal loading rates were 0 (control) and 1000 mg/l. The 1000 mg/l WAF solution was prepared by adding 20.0 g of test substance to 20 liters of dilution water. The mixture was stirred for 24 hours, taking care to avoid the formation of a vortex or gross mixing. After the stirring period, the solution was allowed to settle for 1 hour, then the aqueous phase was removed and dispensed to a 20-liter glass exposure vessel. Duplicate exposure vessels were used for the 1000 mg/l treatment group; a single vessel was used for the control group. The WAFs for each vessel were made independently of each other (i.e., no batch preparations). Each vessel held 10 fish.
Dilution water was dechlorinated laboratory tap water having a total hardness of approximately 100 mg/l as $CaCO_3$.
Rainbow trout were obtained from a commercial supplier (Parkwood Trout Farm, Wigmore, Kent, U.K.) and were maintained in the laboratory approximately 6.5 weeks until use in testing. They were acclimatized to the test condition a week prior to use with no mortality during the acclimation period. During holding and acclimation, fish were fed commercial trout pellets daily up to 24 hour prior to initiation of the test. Fish were not fed during the test. Fish used in the experiment had a mean standard length of

4.8 cm (SD=0.2) and a mean weight of 1.06 g (SD=0.14). The fish biomass loading for the test was 0.53 g/l. Mortality was defined as absence of (1) respiratory movement and (2) response to physical stimulation.
The test was conducted under a photoperiod of 16 h light and 8 h dark. Test solutions were aerated during the test by means of narrow bore glass tubes. The water pH, dissolved oxygen concentration and temperature in each test vessel was recorded daily. Water pH ranged from 7.4 to 7.5, dissolved oxygen ranged from 9.8 to 10.0 mg/l, and temperature remained a constant 14° C. Total organic carbon was measured during the test on samples of fresh (0 and 72 hours) and old (24 and 96 hours) test media.

Reliability : (1) valid without restriction

(67)

Method : Acute toxicity tests
Test substance : Various base oils

Result : The following studies have been added in suporting evidence to the detailed robust summary given above for fish toxicity.
Acute fish toxicity studies have been reported for 14 base oil samples (including the studies summarized in full above). The results for all 14 samples are summarized in the table below.

Result	Reference
Salmo gairdneri - semistatic test	
Distillates, solvent-refined heavy paraffinic (64741-88-4)	
7-d LL_0=1000 ppm dispersion	BP AT301/028
7-d LL_0=1000 ppm dispersion	BP AT301/022
7-d LL_0=1000 ppm dispersion	BP AT301/023
7-d LL_0=1000 ppm dispersion	BP AT301/027
7-d LL_0=1000 ppm dispersion	BP AT301/024
7-d LL_0=1000 ppm dispersion	BP AT301/025
Distillates, solvent refined light paraffinic (64741-89-5)	
96-h LL_0=1000 ppm dispersion	BP AT301/044
7-d LL_0=1000 ppm dispersion	BP AT301/021
Solvent deasphalted bright stock (64741-95-3)	
96-h LL_0=1000 ppm dispersion	BP AT301/043R
Solvent refined residual oil (64742-01-4)	
7-d LL_0=1000 ppm dispersion	BP AT301/026
96-h LL_0=1000 ppm dispersion	BP AT301/042
Pimephales promelas - static test	
Distillates hydrotreated heavy paraffinic (64742-54-7)	
96-h LL_0=100 ppm WAF	EBSI 198140
Solvent dewaxed residual oil (64742-62-7)	
96-h LL_0=100 ppm WAF	EBSI 198240
Distillates solvent dewaxed heavy paraffinic (64742-65-0)	
96-h LL_0=100 ppm WAF	EBSI 101740

(42) (43) (44) (45) (46) (47) (49) (50) (51) (52) (64) (88) (89) (90)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	Static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	No
Year	:	1988
GLP	:	No
Test substance	:	CAS No. 64742-53-6 or 64741-97-5, Distillates (petroleum), hydrotreated or solvent-refined light naphthenic
Result	:	After 48 hrs no daphnid immobilization was found in any of the concentrations tested. The 48 hr EL_0 was 10 g/l. Control survival was 100% after 48 hrs.
Test condition	:	Individual treatment concentrations were prepared as water accommodated fractions (WAF). Nominal loading rates in the definitive test were 0, 0.01, 0.1, 1, and 10 g/l. Control and dilution water was reconstituted hard water prepared by adding salts to glass-distilled deionized water following EPA guidelines (hardness 174 mg/ml as $CaCO_3$). Test substance was mixed in dilution water for 23 hrs. The mixtures were allowed to stand for 1 hr prior to siphoning off the aqueous phase for testing. Glass flasks (140 ml) were filled with each of the WAFs with 10 daphnids per vessel. The flasks were sealed with glass cover slip to minimize the loss of volatile components of the oil. Test daphnids were <24 hrs old and collected from cultures supplied by the testing laboratory that have been aged between 15 and 35 days. Two replicates per treatment and control were used. Black caps were placed over those flasks in which an oily film was visible on the surface of the test solution so the organisms would avoid the darkened zone and not be trapped in the film. Test temperature was 18 - 22 °C. Dissolved oxygen in the control and highest concentration was 8.8 to 9.1 mg/ml. pH in the control and highest concentration was 7.7 - 8.0.
Reliability	:	(2) valid with restrictions Although test guidelines were not specified and the study was not conducted under GLPs, it was a well-documented study. Analytical monitoring of the oil concentration in the WAFs was not performed. An oily film was visible on the surface of some test solutions apparently as a carryover from the WAF preparations.

(118)

Type	:	Semistatic
Species	:	Gammarus pulex (Crustacea)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	No
Year	:	1988
GLP	:	No
Test substance	:	CAS No. 64742-53-6 or 64741-97-5, Distillates (petroleum), hydrotreated or solvent-refined light naphthenic
Result	:	No dead organisms were found in any of the test vessels after 96 hours. However, some organisms disappeared from all treatments and control throughout the test. It was assumed that these organisms were eaten by

the remaining organisms.
The numbers of missing animals after 96 hours were 2, 1, 4, 5, and 2 in the control, 0.01, 0.1, 1, and 10 g/l WAFs. Since <50% of the organisms were missing in any concentration, and even if these lost animals died as a result of treatment, the 96-hr LL_0 was 10 g/l.

Test condition : Individual treatment concentrations were prepared as water accommodated fractions (WAF). Nominal loading rates in the definitive test were 0, 0.01, 0.1, 1, and 10 g/l. Control and dilution water was laboratory mains tap water obtained from bore holes, and passed through particle and activated carbon filters (alkalinity 247 mg/ml as $CaCO_3$, hardness 274 mg/ml as $CaCO_3$, conductivity 492 mS/cm, pH 7.3). Test substance was mixed in dilution water for 23 hrs. The mixtures were allowed to stand for 1 hr prior to siphoning off the aqueous phase for testing. Fresh WAFs were prepared for each 24-hr renewal. Glass crystallizing dishes (350 ml) were filled with 300 ml of each of the WAFs with 10 organisms per dish. Three replicates per treatment and control were used. Test organisms were between 1 and 2 mm in size and collected from a tributary of the River Len at Hollingbourne, Kent, UK. Test temperature was 14 - 18.2 °C.
Dissolved oxygen in the control and highest concentration was 7.8 to 9.9 mg/ml. pH in the control and highest concentration was 6.8 - 8.5.

Reliability : (2) valid with restrictions
Although test guidelines were not specified and the study was not conducted under GLPs, it was a well-documented study. Analytical monitoring of the oil concentration in the WAFs was not performed.

(118)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 1994
GLP : Yes
Test substance : Distillate aromatic extract, CAS No. 64742-04-7

Remark : A 24-hour WAF mixing period was selected based upon a mixing trial with the test substance. No substantial differences in the total organic carbon content in the aqueous phase was seen between 24 and 48-hour mixing periods.

Result : There was no immobilization or other adverse reaction to the exposure solutions during the test. Inspection of the data revealed the following:
48-H EL_{50} = >1000 mg/l WAF
Highest test concentration resulting in 0% immobilization:
1000 mg/l WAF
Lowest test concentraiton resulting in 100% immobilization:
> 1000 mg/l WAF
No Observed Effect Level (NOEL): 1000 mg/l WAF

Total organic carbon analyses (mg/l):

Treatment Group	0-h	48 h
Control	3.587	2.256
1000 mg/l R1 and R2	1.937	1.997
1000 mg/l R3 and R4	2.168	1.831

Total organic carbon measurements made on the exposure solutions during the test were variable. The authors claim that the carbon analyses

do not provide definitive evidence of stability of the test preparations.

Test condition : A static 48-hour toxicity test was conducted without renewal of test solutions. Test solutions were prepared as water accommodated fractions (WAF). Nominal loading rates were 0 (control) and 1000 mg/l. The 1000 mg/l WAF solution was prepared by adding 2 g of test substance to 2 liters of dilution water. The mixture was stirred for 24 hours, taking care to avoid the formation of a vortex or gross mixing. After the stirring period, the solution was allowed to settle for 1 hour, then the aqueous phase was removed and 200 ml of the solution was dispensed into each of four replicate glass vessels. The 1000 mg/l WAF treatment used four replicate vessels, while the control treatment used two replicate vessels. Each vessels held 10 daphnids, and all vessels were covered during the test to reduce evaporation.
Dilution water was reconstituted water having a total hardness of approximately 270 mg/l as $CaCO_3$.
Daphnids used in the test had been cultured at 21 °C in the laboratory in reconstituted water. The original culture was obtained from the Institut National de Recherche Chimique Appliquee, France. Cultures were fed daily with a suspension of mixed algae (predominately Chlorella sp.). Gravid adults were isolated 24 hours prior to initiation of the test, and the young daphnids produced overnight were used for testing. The daphnid loading rate during the test was 20 ml solution per daphnid. Immobilization was defined as the inability to swim for approximately 15 seconds after gentle agitation.
The test was conducted under a photoperiod of 16 h light and 8 h dark. No aeration was applied during the test. Temperature was recorded daily, and pH and dissolved oxygen were recorded at initiation and termination of the test. Water pH ranged from 7.7 to 7.9, dissolved oxygen ranged from 7.8 to 8.1, and temperature remained a constant 21 °C. Total organic carbon was measured as a means to demonstrate stability of the test solutions. Measurements were made of test solutions collected at 0 and 48 hours.

Reliability : (1) valid without restriction

(66) (82)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Scenedesmus subspicatus (Algae)
Endpoint	:	growth rate
Exposure period	:	96 hour(s)
Unit	:	mg/l
Limit test	:	Yes
Analytical monitoring	:	Yes
Method	:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year	:	1991
GLP	:	Yes
Test substance	:	CAS No. 64741-88-4; distillates (petroleum), solvent-refined, heavy paraffinic

Result : No inhibition of growth or growth rate were measured at the single test concentration of 50% WAF. Since there were no observed effects during the study, the 96-hour "No Observed Effect Concentration" (NOEC) was 50% WAF. The 50% WAF solution was equal to a test substance loading rate of 500 mg/l.
The OECD guideline criterion for cell growth in the control group was met in this experiment.

Test condition : Preparation of the Water Accommodated Fraction (WAF):2.0 grams of test

material were placed on 2 Liters of culture medium and stirred via magnetic stirrer for a period of 24 hours prior to the test. Culture medium was prepared according to the guideline formula. After the 24 hour period, stirring was ceased for one hour prior to removing the aqueous phase. The aqueous phase, representing 100% WAF, was then combined with an equal volume of algal suspension. The algal suspension consisted of Scenedesmus cells taken from a culture in logarithmic growth phase and diluted with growth medium to a cell density of 3.70×10^4 cells/ml. The algal species Scenedesmus subspicatus utilized in this study was supplied by the Culture Centre of Algae and Protozoa (CCAP) c/o Institute of Freshwater Ecology, Cumbria, U.K. Sterile culture medium was inoculated with Scenedesmus and incubated under continuous illumination and aeration at 21°C.
10 ml samples of the 50% WAF were taken at times 0 and 96 hours. After adding 10 ml of 1,1,2-trichlorotrifluoroethane, the samples were stored at 4°C until analyzed. Analytical results were not reported.
500 ml of the algal suspension were added to 500 ml of 100% WAF to make the test solution. 100 ml of the test solution was contained in a loosely stoppered 250 ml conical flask. All flasks were incubated and shaken at approximately 100 rpm in an orbital shaker. 6 replicates of a single test concentration and 3 replicates of a control were examined in this study. The flasks were housed under a 24 hour light photoperiod at an intensity of approximately 7,000 lux and a constant temperature of 24°C. No aeration was supplied during the study, however, gas exchange and algal cell suspension was maintained by the orbital shaker. Samples were taken for the determination of algal growth every 24 hours beginning at hour 0 and ending at hour 96. Absorbances were measured at 665 nm with a Jenway 610 Spectrophotometer. At the initiation and completion of the experiment, the cell densities of the control cultures were determined through direct counting aided by a hemacytometer. The pH of all control and test flasks was taken at 0 and 96 hours. The pH at the beginning and end of the experiment in all groups ranged from 8.3 to 8.5 and 9.4 to 9.9, respectively. The area under the curve and growth rate were taken as indices of algal growth and were calculated using the absorbance readings. Percent inhibition values were calculated for area under the curve and growth rate.

Remark : Three other base oil samples have been tested for algal toxicity. The results for all three samples were similar to that described above. Samples tested at one concentration only were as follows:

CAS No.	Result	Ref.
64741-88-4	96-h LL_0 = 50% WAF	BP Project 301/74
64741-89-5	96-h LL_0 = 50% WAF	BP Project 301/70
64742-01-4	96-h LL_0 = 50% WAF	BP Project 301/76

Reliability : (2) valid with restrictions
Only one concentration of the test substance was tested. Results of chemical analyses of test substance concentrations were not reported.
(38) (39) (40) (41)

Species : Scenedesmus subspicatus (Algae)
Exposure period : 72 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 1994
GLP : Yes

Test substance : Distillate aromatic extract, CAS No. 64742-04-7

Method : Statistical method: One-way analysis of variance

Remark : A 24-hour WAF mixing period was selected based upon a mixing trial using a similar product. No substantial differences in the total organic carbon content in the aqueous phase was seen between 24 and 48-hour mixing periods.

Result : $EbLR_{50}$ (72-h) = >1000 mg/l WAF
$ErLR_{50}$ (24-48 h) = >1000 mg/l WAF
No Observed Effect Level (NOEL) = 1000 mg/l WAF

Results of Absorbance Readings:

	Absorbance values (mean)			
Loading Rate	**0-h**	**24-h**	**48-h**	**72-h**
0 (Control)	0.026	0.043	0.333	0.574
1000 mg/l WAF	0.026	0.045	0.338	0.590

Results of Percent Inhibition Calculations:

	Percent Inhibition Values			
Loading Rate	**AUGC (72-h)**	**% Inhibition**	**Growth Rate (24-48 h)**	**% Inhibition**
Control	14.372	--	0.085	--
1000 mg/l WAF	14.706	-2	0.084	1

Results of Total Organic Carbon analyses (mg/l):

Loading Rate	**0-h**	**72 h**
0 (control)	23.27	4.636
1000 mg/l WAF	10.16	5.215

Total organic carbon measurements made on the exposure solutions during the test were variable. The authors claim that the carbon analyses do not provide definitive evidence of stability of the test preparations.

Test condition : A 72-h static toxicity test was conducted without renewal of test solutions. Test solutions were prepared as water accommodated fractions (WAF). Nominal loading rates were 0 (control) and 1000 mg/l WAF. The 1000 mg/l WAF solution was made by adding 4 g of test substance in 2 liters of algal culture medium to give a loading rate of 2000 mg/l. The mixture was stirred for 24 hours, taking care to avoid the formation of a vortex or gross mixing. After the stirring period, the solution was allowed to settle for 1 hour, then the aqueous phase was removed. The 2000 mg/l WAF was diluted 50:50 with an algal suspension to create a 1000 mg/l WAF.
Algal culture medium was prepared according to the recipe given in OECD Guideline 201.
Scenedesmus subspicatus cultures originated from the Culture Centre of Algae and Protozoa (CCAP), Institute of Freshwater Ecology, Cumbria, U.K. The algal suspension used in the test was prepared by first inoculating sterile culture medium with S. subspicatus taken from a master culture. The suspension was incubated at 21 °C under continuous illumination of approximately 7000 lux until reaching log-phase growth, which was characterized by an absorbance of 0.780 (@665 nm). 300 ml of the suspension was added to 300 ml of the 2000 mg/l WAF solution to achieve 600 ml of 1000 mg/l WAF test solution. This solution had an absorbance of 0.026 and a mean cell density of 3.69×10^4 cells/ml at the start of the test.
Test vessels were 250-ml conical flasks holding 100 ml of test solution. They were loosely stoppered to reduce evaporation. Six replicate flasks of inoculated 1000 mg/l WAF solution and three replicate flasks holding inoculated control medium were prepared and incubated for 72 hours

under continuous lighting at approximately 24 °C. Separate flasks holding culture medium and 1000 mg/l WAF solution were similarly held and used for total organic carbon analysis at 0 and 72 hours. The pH of the test and control solutions was measured at 0 and 72 hours. Test solution and control solution pH values at 0 and 72 hours ranged 8.0 to 10.0 and 8.0 to 9.8, respectively.
Samples were taken from each flask at 0, 24, 48 and 72 hours, and the absorbance at 665 nm was measured using a Jenway 6100 Spectrophotometer. Cell densities of the control cultures at 0, 24, 48 and 72 hours were measured by direct counting with the aid of a haemocytometer to confirm that absorbance values were well correlated with cell densities to be used to monitor the growth of the test cultures. Area under the growth curve (AUGC) was used as an index of growth, and percent inhibition of the AUGC and percent inhibition of growth rate were used to assess effects of the test substance. The AUGC, average maximum growth rates and the percent inhibition of the AUGC and growth rates were calculated according to OECD Guideline 201. The effective loading rate for biomass ($EbLR_{50}$) and growth rate ($ErLR_{50}$) were evaluated using the inhibition data.

Reliability : (1) valid without restriction

(65) (82)

Species	:	Scenedesmus subspicatus (Algae)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	Yes
Method	:	Directive 87/302/EEC, part C, p. 89 "Algal inhibition test"
Year	:	1991
GLP	:	Yes
Test substance	:	Solvent-refined residual oil, CAS No. 64742-01-4

Result : No inhibition of growth or growth rate were measured at the single test concentration of 50% WAF. The 50% WAF solution was equal to a test substance loading rate of 500 mg/l.

Since there was neither a 50% decline in biomass, nor a 50% decline in growth rate, the 96-hour EbC_{50} and the 0-24 hour ErC_{50} are reported as being greater than 50% WAF. Since there were no observed effects during the study, the "No Observed Effect Concentration" (NOEC) for the algae exposed to the test material is reported as being equal to 50% WAF.
The OECD guideline criterion for cell growth in the control group was met in this experiment.

Test condition : Preparation of the Water Accommodated Fraction (WAF):
2.0 grams of test material were placed on 2 liters of culture medium and stirred via magnetic stirrer for a period of 24 hours prior to the test. After the 24 hour period, stirring was ceased for one hour prior to removing the aqueous phase. The aqueous phase, representing 100% WAF, was then combined with an equal volume of algal suspension.
10 ml samples of the 50% WAF were taken at times 0 and 96 hours. After adding 10 ml of 1,1,2-trichlorotrifluoroethane, the samples were stored at 4°C until analyzed by the sponsor. Results of any analyses were not reported.

Culture Meduim:
15 mg/l NH_4Cl
12mg/l $MgCl_2*6H_2O$
18mg/l $CaCl_2*2H_2O$

15 mg/l $MgSO_4*7H_2O$
1.6 mg/l KH_2PO_4
0.08 mg/l $FeCl_3*6H_2O$
0.1 mg/l $NA_2EDTA*2H_2O$
0.185 mg/l H_3BO_3
0.415 mg/l $MnCl_2*4H_2O$
$3x10^{-3}$ mg/l $ZnCl_2$
$1.5x10^{-3}$ mg/l $CoCl_2*6H_2O$
10^{-5} mg/l $CuCl_2*2H_2O$
$7x10^{-3}$ mg/l $Na_2MoO_4*2H_20$
50 mg/l $NaHCO_3$

The algal species Scenedesmus subspicatus utilized in this study was supplied by the Culture Centre of Algae and Protozoa (CCAP) c/o Institute of Freshwater Ecology, Cumbria, U.K. Sterile culture medium was inoculated with Scenedesmus and incubated under continuous illumination and aeration at 24°C. This produced an algal suspension in log phase growth characterized by an absorbance of 0.451 (at 665 nm). Prior to use, the suspension was diluted to an absorbance of 0.022, yielding a mean cell density of 3.69×10^4 cells/ml.

100 ml of the test solution was contained in a loosely stoppered 250 ml conical flask. All flasks were incubated and shaken at approximately 100 rpm in an orbital shaker. 6 replicates of a single test concentration and 3 replicates of a control were examined in this study. 500 ml of the algal suspension were added to 500 ml of 100% WAF to make the test solution. The flasks were housed under a 24 hour light photoperiod at an intensity of approximately 7,000 lux and a constant temperature of 24 °C. No aeration was supplied during the study, however, gas exchange and algal cell suspension was maintained by the orbital shaker. Samples were taken for the determination of algal growth every 24 hours beginning at hour 0 and ending at hour 96. Absorbances were measured at 665 nm with a Jenway 610 Spectrophotometer. At the initiation and completion of the experiment, the cell densities of the control cultures were determined through direct counting aided by a haemacytometer. The pH of all control and test flasks was taken at 0 and 96 hours. The pH at the beginning and end of the experiment in all groups ranged from 8.0 to 8.1 and 9.6 to 10.0, respectively.

The area under the curve and growth rate were taken as indices of algal growth and were calculated using the absorbance readings:

$$A = (N1 - N2)/2 \times t1 + (N1+N2 - 2N0)/2 \times (t2 - t1) + (Nn-1 + Nn - 2N0)/2 \times (tn - tn-1)$$

A= area
N0 = absorbance at t0
N1 = absorbance at t1
Nn = absorbance at tn
t1 = time of first measurment (hours from start)
tn = time of nth measurement (hours from start)

Growth Rate: (determined only for 0 - 24 hour period)

$$u = (\ln Nn - \ln N1)/(tn - t1)$$

Calculation of Inhibition:
Percentage inhibition of growth (IA) and growth rate (Iu) were calculated by the following equations:
IA = (Ac - At)/Ac x 100
and
Iu = (uc - ut)/uc x 100

Reliability : (2) valid with restrictions
Only one concentration of the test substance was tested. Results of chemical analyses of test substance concentrations were not reported.
(27)

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species	:	Daphnia magna (Crustacea)
Exposure period	:	21 day(s)
Unit	:	mg/l
Analytical monitoring	:	Yes
Method	:	OECD Guide-line 202, part 2 "Daphnia sp., Reproduction Test"
Year	:	1995
GLP	:	Yes
Test substance	:	CAS No. 64741-88-4; distillates (petroleum), solvent-refined, heavy paraffinic

Result : After 14 and 21 days of exposure, there were no statistically significant differences between the control group and the 10 and 1000 mg/ml WAF test groups in terms of survival or reproduction (young produced per adult). In addition, there were no apparent effects on the F1 generation produced during the test. The numbers of unhatched eggs and dead young were low in all treatment groups.

The NOEC for survival and reproduction was the maximum test concentration, 1000 mg/ml WAF.

The test met the validation criteria for 1) dissolved oxygen at least 60%, 2) pH deviation not greater than 0.3, 3) control mortality not greater than 20%, 4) first young (control group) within 9 days, 5) cumulative young per female (control group) at least 20 after 14 days and at control group at least 3.

Test condition : Preparation of the WAF:
20 and 2000 mg of test material were each separately placed in 2 liters of reconstituted water (water hardness approximately 270 mg/ml as $CaCO_2$) and stirred via magnetic stirrer for a period of 24 hours prior to the test. After the 24-hour period, stirring was ceased for one hour prior to removing the aqueous phase.

Test Organism Culture:
Adult Daphnia magna were maintained in polypropylene vessels containing approximately 2 liters of reconstituted water at a temperature of 21°C. The organisms were supplied by the Institut National de Recherche Appliquée (IRCHA) France. The lighting was held at 16:8 hour light:dark photoperiod. Gravid adults were isolated 24 hours prior to the initiation of the test, the young daphnids produced overnight were removed and utilized for testing.

Test Procedure:
The aqueous phase of each WAF was removed and 400-ml aliquots were

apportioned to five, 500-ml glass flasks. A similar number of control flasks containing reconstituted water also were prepared. The fifth flask from each group was taken for Total Organic Carbon analysis of the exposure media. At the start of the test, 10 daphnids were placed within each test flask, and all flasks were covered to reduce evaporation. Each vessel received approximately 3.75 x 10^9 cells/ml of a mixed unicellular algae culture as a daily feeding. Fresh WAFs were prepared on days 0, 2, 4, 7, 9, 11, 14, 16, and 18, and the adult daphnids were transferred from the old to the fresh solutions. The numbers of live and dead Daphnia of the parental generation were counted daily. At each test media renewal, Daphnia with eggs or young in the brood pouch, discarded unhatched eggs, and the number of live and dead filial Daphnia were counted. Temperature was recorded daily for the duration of the experiment, while dissolved oxygen and pH were recorded prior to and after each media renewal. Measurements of TOC were made in the fresh and old test solutions 3 times a week over 21 days. Dissolved oxygen in the control, 10, and 1000 mg/ml WAF groups ranged from 7.9 to 8.3, from 7.9 to 8.3, and from 7.8 to 8.3, respectively. Water pH in the control, 10, and 1000 mg/ml WAF groups ranged from 7.7 to 7.8, from 7.7 to 7.8, and from 7.7 to 7.8, respectively. The temperature within all test groups remained constant at 21.0 °C. The results of the TOC analysis did not demonstrate a cases the TOC of the control water was higher than that of the test groups. The TOC in the old media tended to be higher than fresh solutions.

Reliability : (2) valid with restrictions
The analytical results provided no definitive evidence of stability of the test preparations. Only two test concentrations were run.

(71)

Species : Daphnia magna (Crustacea)
Exposure period : 21 day(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 202, part 2 "Daphnia sp., Reproduction Test"
Year : 1995
GLP : Yes
Test substance : Solvent-refined residual oil, CAS No. 64742-01-4

Result : After 14 and 21 days of exposure, there were no statistically significant differences between the control group and the 10 and 1000 mg/l WAF test groups in terms of survival or reproduction (young produced per adult). In addition, there were no apparent effects on the F1 generation produced during the test. The numbers of unhatched eggs and dead young were low in all treatment groups.

The NOEC for survival and reproduction was the maximum test concentration, 1000 mg/l WAF.

The test met the validation criteria for
1) dissolved oxygen >60%
2) pH deviation W0.3
3) control mortality W
4) first young (control group) within 9 days
5) cumulative young per female (control group) D20 after 14 days and D40 after 21 days
6) number of broods per control group D3.

Test condition : Preparation of Reconstituted Water:
Reconstituted water was prepared by combining 25 ml of each of the

following stock solutions and bringing to a volume of 1 Liter with deionized water (conductivity <5 S cm^{-1}; pH = 7.8 0.2). The reconstituted water was aerated until the dissolved oxygen was approximately air saturation. The reconstituted water as prepared had a total hardness of approximately 270 mg/l as $CaCO_3$.
1) 11.76 g/l $CaCl_2.2H_2O$
2) 4.63 g/l $MgSO_4.7H_2O$
3) 2.59 g/l $NaHCO_3$
4) 0.23 g/l KCl

Preparation of the WAF:
20 and 2000 mg of test material were each separately placed in 2 liters of reconstituted water and stirred via magnetic stirrer for a period of 24 hours prior to the test. After the 24-hour period, stirring was ceased for one hour prior to removing the aqueous phase.

Test Organism Culture:
Adult Daphnia magna were maintained in polypropylene vessels containing approximately 2 liters of reconstituted water at a temperature of 21 °C. The organisms were supplied by the Institut National de Recherche Appliquée (IRCHA) France. The lighting was held at at 16:8 hour light:dark photoperiod. Gravid adults were isolated 24 hours prior to the initiation of the test, the young daphnids produced overnight were removed and utilized for testing.

Test Procedure:
The aqueous phase of each WAF was removed and 400-ml aliquots were apportioned to five, 500-ml glass flasks. A similar number of control flasks containing reconstituted water also were prepared. The fifth flask from each group was taken for Total Organic Carbon analysis of the exposure media. The four remaining flasks of each group were used to hold test daphnids. At the start of the test, 10 daphnids were placed within each test flask, and the flasks were covered to reduce evaporation. Fresh WAFs were prepared on days 0, 2, 4, 7, 9, 11, 14, 16, and 18, and the adult daphnids were transferred from the old to the fresh solutions. The old solutions were strained through a fine mesh and any retained young daphnids (live or dead) and unhatched eggs were counted using a stereo microscope prior to being discarded.

Each vessel received approximately 3.75×10^9 cells/ml of a mixed unicellular algae culture as a daily feeding. This level allowed for continuos feeding throughout the experiment. The numbers of live and dead Daphnia of the parental generation were counted daily. At each test media renewal, Daphnia with eggs or young in the brood pouch, discarded unhatched eggs, and the number of live and dead filial Daphnia were counted.

Temperature was recorded daily for the duration of the experiment, while dissolved oxygen and pH were recorded prior to and after each media renewal. Measurements of TOC were made in the fresh and old test solutions. Dissolved oxygen in the control, 10, and 1000 mg/L WAF groups ranged from 7.9 to 8.5, from 7.9 to 8.5, and from 7.9 to 8.5, respectively. Water pH in the control, 10, and 1000 mg/l WAF groups ranged from 7.6 to 7.8, from 7.6 to 7.8, and from 7.6 to 7.8, respectively. The temperature within all test groups remained constant at 21.0 °C. The results of the TOC analysis did not demonstrate a direct relationship with WAF concentration, and in many cases the TOC of the control water was higher than that of the test groups. The TOC in the old media tended to be higher than fresh

solutions.

Analytical Monitoring:
The stability of the test material (both freshly renewed and old) in the test solutions was verified by Total Organic Carbon (TOC) analysis of the control and the WAF loaded groups 3 times per week over 21 days.

Reliability : (2) valid with restrictions
The analytical results provided no definitive evidence of stability of the test preparations

(68) (79) (84) (108) (121) (125) (129)

Exposure period : 21 day(s)
Unit : mg/l

Result : In addition to the studies described above, studies have been reported for nine further base oil samples in 21 day studies with D. magna. These have been added as supporting evidence to the detailed robust summaries given above for chronic toxicity to aquatic invertebrates. In each case OECD guideline 202 part 2 was used as the method.
The results are summarized below:

CAS No.	Result	Reference
64741-88-4	21-d LL_0 = 1000 mg/l WAF	BP 692/039
64741-88-4	21-d LL_0 = 1000 mg/l WAF	BP 692/040
64741-88-4	21-d LL_0 = 1000 mg/l WAF	Shell Exp. 5922
64741-89-5	21-d LL_0 = 1000 mg/l WAF	BP 692/036
64741-89-5	21-d LL_0 = 1000 mg/l WAF	BP 692/037
64741-95-3	21-d LL_0 = 1000 mg/l WAF	BP 692/042
64742-01-4	21-d LL_0 = 1000 mg/l WAF	BP 692/041
64742-53-6	21-d LL_0 = 10 mg/l WAF	Shell Exp. 6215
64742-55-8	21-d LL_0 = 1000 mg/l WAF	Shell Exp. 5922
64742-65-0	21-d LL_0 = 1000 mg/l WAF	Shell Exp. 5922

(70) (72) (73) (74) (75) (76) (114) (115)

Species : Daphnia magna (Crustacea)
Exposure period : 21 day(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 202, part 2 "Daphnia sp., Reproduction Test"
Year : 1995
GLP : Yes
Test substance : Distillate aromatic extract, CAS N. 64742-04-7

Remark : A 24-hour WAF mixing period was selected based upon a mixing trial using a similar product. No substantial differences in the total organic carbon content in the aqueous phase was seen between 24 and 48-hour mixing periods.

Result : Summary of Findings:

	Nominal loading rate (mg/l)		
	0	10	1000
% survival of parental generation	100	100	100
No live young			
Total	2105	2046	2108
per Female	53	51	53

No. dead			
Total	0	0	0
per Female	0	0	0
No. unhatched eggs			
Total	2	1	0
per Female	<1	<1	0

Lethal Effects on Parental Generation:
21 d ELR_{50} (survival) = >1000 mg/l WAF

Sublethal Effects on Parental Generation:
21-d ELR_{50} (reproduction) = >1000 mg/l WAF

Effects on Filial (F1) Generation: No discernable effects noted.

No Observed Effect Level (NOEL) for the Test:
NOEL = 1000 mg/l WAF

Ranges of TOC Measurements (mg C/l):
Nominal

Loading Rate (mg/l)	**Fresh Solutions**	**Old Solutions**
0 (control)	1.243 - 3.161	1.438 - 3.645
10	1.492 - 5.149	0.635 - 2.753
1000	1.608 - 3.975	1.109 - 5.181

The author's claim that the total organic carbon measurements made on the control and test solutions were variable and tended to approximate the detection limit. Furthermore, the carbon analyses do not provide definitive evidence of stability of the test preparations.

Validation Criteria:
All validation criteria were met for the test. These criteria included:
1) control mortality 20%
2) dissolved oxygen concentration 60% saturation
3) pH deviation 0.3
4) time to production of first young in control group 9 days
5) cumulative young produced per female in control group
   20 @ 14 d;
   40 @ 21 d
6) number of broods per control group 3

Test condition : A semi-static 21-day chronic toxicity test was conducted with renewal of test solutions three times per week. Test solutions were prepared as water accommodated fractions (WAF). Nominal loading rates were 0 (control), 10, and 1000 mg/l. The 10 and 1000 mg/l WAF solutions were prepared by adding 0.02 and 2 g, respectively of test substance to 2 liters of dilution water. The mixtures were stirred for 24 hours, taking care to avoid the formation of a vortex or gross mixing. After the stirring period, the solutions were allowed to settle for 1 hour, then the aqueous phase of each was removed and dispensed into replicate glass test vessels. Glass flasks served as replicate test vessels with each replicate holding 400 ml of test solution. There were four replicate test vessels per treatment and each vessel contained 10 dapnids at test initiation. A fifth replicate of each test level was prepared and was used for sampling for total organic carbon (TOC) analyses.
Dilution water was reconstituted freshwater having a total hardness of approximately 270 mg/l as $CaCO_3$.

Daphnids used in the test had been cultured at 21 °C in the laboratory in reconstituted water. The original culture was obtained from the Institut National de Recherche Chimique Appliquee, France. Cultures were fed daily with a suspension of mixed algae (predominately Chlorella sp.). Gravid adults were isolated 24 hours prior to initiation of the test, and the young daphnids produced overnight were used for testing. The daphnid loading rate during the test was 40 ml solution per daphnid. Daphnids were fed daily 10 l of a mixed unicellular algal suspension (equivalent to 3.3×10^9 cells/ml and 0.24 mg C/daphnid/day). Live and dead daphnids of the parental generation were counted daily. At each renewal period (three times per week), the general condition and size of parental generation daphnids were evaluated, and the numbers of adults with eggs or young in the brood pouch, numbers of live and dead F1 generation daphnids, and the numbers of discarded unhatched eggs were determined. At the renewal periods, adult daphnids were transferred to fresh media by wide-bore pipette then the contents of each vessel were passed through a fine mesh. Young daphnids (live and dead) and unhatched eggs were collected in this manner and counted. Young daphnids were considered dead if no sign of movement was apparent during microscopic examination. Adult daphnids which were unable to swim for approximately 15 seconds after gentle agitation were considered dead.
The test was conducted under a photoperiod of 16 h light and 8 h dark and 21 ³C. No aeration was applied during the test. Temperature was recorded daily, and dissolved oxygen, pH and temperature were recorded before and after each renewal period. TOC analyses were carried out on fresh test solutions on days 0, 2, 5, 7, 9, 12, 14, 16, and 19, and on old solutions on days 2, 5, 7, 9, 12, 14, 16, 19, and 21. Water quality in the fresh and old solutions remained consistent during the test. The pH of fresh and old solutions ranged from 7.7 to 7.9, dissolved oxygen ranged from 7.8 to 8.4 mg O_2/l, and temperature remained a constant 21.0 °C.

Reliability : (1) valid without restriction

(69) (82)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD_{50}
Value	:	> 5000 mg/kg bw
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Male/female
Number of animals	:	5
Vehicle	:	None - administered undiluted
Year	:	1986
GLP	:	Yes
Test substance	:	Unrefined base oil Sample API 84-01 [CAS 64741-50-0] See section 1.1.1.
Method	:	A single dose of undiluted test material (5g/kg) was administered orally to 5 male and 5 female fasted rats. Food and water was made available ad-lib immediately after dosing. The animals were observed for clinical signs and mortality at hourly intervals for the first 6 hours post dosing and twice daily thereafter. Body weights were recorded prior to fasting, prior to dosing and at 7 and 14 days post dosing. At 14 days, all surviving animals were killed and subjected to a gross necropsy examination.
Result	:	There were no deaths during the study and growth rates were unaffected by dosing. Clinical signs that occurred during the first 3 days included: hypoactivity, diarrhea and a yellow-stained anal area. All animals returned to normal by day 14. At gross necropsy, there were no visible lesions.
Reliability	:	(1) valid without restriction

(13)

Type	:	LD_{50}
Value	:	> 5000 mg/kg bw
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Male/female
Number of animals	:	5
Vehicle	:	None - administered undiluted
Year	:	1986
GLP	:	Yes
Test substance	:	Highly refined Base oil Sample API 83-12 [CAS64742-53-6] See section 1.1.1.
Method	:	A single dose of undiluted test material (5g/kg) was administered orally to 5 male and 5 female fasted rats. Food and water was made available ad-lib immediately after dosing. The animals were observed for clinical signs and mortality at hourly intervals for the first 6 hours post dosing and twice daily thereafter. Body weights were recorded prior to fasting, prior to dosing and at 7 and 14 days post dosing. At 14 days, all surviving animals were killed and subjected to a gross necropsy examination.
Result	:	There were no deaths during the study. Clinical signs observed included: hypoactivity, yellow-stained anal area, hair loss in the urogenital region and swollen hind paws. All animals returned to normal by day 3 and had gained weight by day 7. At necropsy, there were no visible lesions except in one female in which the spleen was cystic, mottled red and tan and had a rough surface. In this animal the

pancreas adhered to the entire surface of the spleen.

Reliability : (1) valid without restriction

(12)

Type : LD_{50}
Species : Rat
Test substance : Various Base oils

Remark : CONCAWE summarized the data available on the acute oral toxicity of lubricating oil base stocks. The data are shown in the following table.

	CAS No.	Oral LD50 (g/kg)	API Report No.
Paraffinic distillates			
Solvent dewaxed, light			
API 78-9	64742-56-9	>5	29-33104
Solvent dewaxed, heavy			
API 78-10*	64742-56-0	>5	29-33105
API 79-3	64742-65-0	>5	29-33067
API 79-4	64742-65-0	>5	29-33066
API 79-5	64742-65-0	>5	29-33068
White mineral oil			
Tufflo 6056*		>5	39-31651
Naphthenic distillates			
Solvent refined, light			
API 78-5	64741-97-5	>5	29-33106
Solvent refined, heavy			
API 79-1	64741-96-4	>5	29-33065
Hydrotreated, heavy			
API 83-15	64742-52-5	>5	33-32639

* Although these materials are not included in the HPV Lubricating base stocks category, they are similar to other materials in the category and provide supportive information.

(2) (3) (4) (5) (6) (7) (8) (14) (81)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC_{50}
Value : 2.18 mg/l
Species : Rat
Strain : Sprague-Dawley
Sex : Male/female
Number of animals : 5
Vehicle : Air
Exposure time : 4 hour(s)
Year : 1987
GLP : Yes
Test substance : Highly refined Base oil Sample API 83-12 [CAS64742-53-6] See section 1.1.1.

Method : A group of 5 male and 5 female rats were exposed for 4 hours to an aerosol of the test material at a target concentration of 5 mg/l. Four additional groups of rats were then exposed for 4 hours to target aerosol concentrations of 1, 1.5, 2.5 and 3.5 mg/l. A control group exposed, in the

chamber, to air only was also included.
Animals were observed continuously during the first hour of exposure, hourly for the remainder of the exposure and once daily for the 14-day post exposure period. Mortalities were recorded and body weights were measured prior to exposure and again 7 and 14 days after exposure. On the 14th day post-exposure, necropsies were performed on all surviving animals. For all animals, including animals found dead, the lungs and any other abnormal tissues were removed and fixed for subsequent histopathological examination.

Result : Actual exposure concentrations and mortalities were as follows:

Target level (mg/l)	Actual concentration mg/l	±SD	Mortality Male	Female
0	0.02	0.01	0/5	0/5
1.0	1.04	0.1	1/5	1/5
1.5	1.51	0.15	0/5	0/5
2.5	2.37	0.31	3/5	3/5
3.5	3.49	0.36	5/5	5/5
5.0	5.05	0.18	5/5	5/5

Particle size measurements confirmed that mass median aerodynamic diameter and geometric standard deviation values were in the ranges 1.7 to 2.5 mμ and 1.5 to 1.61 respectively. These measurements confirm that the particles were within the respirable range.
The LC_{50} for combined sexes was estimated to be 2.18 with 95% confidence limits of 1.80 to 2.55 mg/l.
Body weight differences did not show a consistent dose related pattern.
At the highest concentration, the animals were obscured by a dense aerosol and observations could not be made during the exposure period. In other groups, there was a decreased activity, wet inguinal area, eyes partially closed, wet coat, loose stool and oily coat during exposure. During the first week post-exposure, similar signs were observed as well as signs of poor condition, respiratory distress and some deaths occurred. During test week 2, most survivors were considered to be of normal appearance. The signs that were observed occurred in a dose related manner.
At gross necropsy, dark red lungs were described for some animals. The incidence is shown below.

Dose group	Male	Female
0	0/5	0/5
1.0	1/5	1/5
1.5	0/5	0/5
2.5	3/5	3/5
3.5	5/5	5/5
5.0	5/5	5/5

At histology, affected animals exhibited diffuse pulmonary congestion and perivascular edema that were mostly moderate or marked in degree. Less consistently spotty alveolar edema was also seen. There was widespread damage to alveolar walls resulting in fibronecrotic debris resembling hyaline membranes in more marked cases and extravasation of RBCs and PMNs. Necrosis and inflammation were seen in the walls of small blood vessels and there was spotty epithelial necrosis in small bronchioles, but the most severe damage seemed to be centroacinar. The larger airways were relatively unaffected.
None of the surviving animals exhibited the above acute changes. However, most of the surviving animals exposed to 2.5 or 1.0 mg/l and

above exhibited chronic inflammatory changes that were not seen in the controls and only occasionally in animals exposed at the 1.5 mg/l level, and then to a lesser degree of severity. Other findings were considered sporadic or unrelated to exposure to the test material.

Test condition : Whole body exposures were carried out in stainless steel and glass chambers of 0.25 cubic meter volume. Aerosols were generated using a nebulizer. Concentrations of test material in the exposure chambers were determined gravimetrically by collection of the aerosol on filters. Analytical samples were taken at least once per hour during the exposure period. Particle size determinations were also carried out.

Reliability : (1) valid without restriction

(16)

Type : LC_{50}
Species : Rat
Test substance : Various Base oils

Remark : CONCAWE summarized the data available on the acute inhalation toxicity of lubricating oil mists in 4 hour exposure studies in rats.
The data (Original source Whitman et al, 1989) on 3 paraffinic distillates are shown in the following table.

	Inhalation LC_{50} (mg/l)
Paraffinic distillates	
Solvent extracted, dewaxed	>4
Solvent extracted, dewaxed, hydrotreated	>4
Solvent dewaxed, light	>4

(81) (126)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD_{50}
Value : > 2000 mg/kg bw
Species : Rabbit
Strain : New Zealand white
Sex : Male/female
Number of animals : 4
Vehicle : None applied undiluted
Year : 1986
GLP : Yes
Test substance : Unrefined base oil Sample API 84-01 [CAS 64741-50-0] See section 1.1.1.

Method : Undiluted test material was applied as a single dose (2g/kg) to the shorn, abraded skin of 4 male and 4 female rabbits. The treated site was covered with an occlusive dressing for 24 hours. After removal of the dressing, the skin was wiped with a wet towel to remove residual test material. The rabbits were observed for clinical signs and mortality hourly for the first 6 hours, then daily for derma irritation and twice daily for clinical signs and mortality.
Observation was carried out for a 14-day post treatment period. Body weights were recorded prior to administration of the test material, again 7 days post dosing and at study termination (14 days). At termination, all surviving animals were killed and subjected to a gross necropsy examination.

Result : There were no mortalities during the study.

With the exception of skin irritation, there were no clinical signs of toxicity except that on day 4 soft stool was observed in 1 male and 3 female animals. Dermal irritation ranged from slight to severe for erythema and edema, from slight to marked for fissuring and slight to moderate for atonia and desquamation. Slight coriaceousness was also observed.
Body weight losses were recorded for 2 male and 3 female animals at day 7. One male was less than starting weight on both day 7 and day 14.

Reliability : (1) valid without restriction

(13)

Type : LD_{50}
Value : > 2000 mg/kg bw
Species : Rabbit
Strain : New Zealand white
Sex : Male/female
Number of animals : 2
Vehicle : None - applied undiluted
Year : 1986
GLP : yes
Test substance : Highly refined Base oil Sample API 83-12 [CAS64742-53-6] See section 1.1.1.

Method : Undiluted test material was applied as a single dose (2g/kg) to the shorn, abraded skin of 4 male and 4 female rabbits. The treated site was covered with an occlusive dressing for 24 hours. After dressing removal, the skin was wiped with a wet towel to remove residual test material. The rabbits were observed for clinical signs and mortality hourly for the first 6 hours, then daily for dermal irritation and twice daily for clinical signs and mortality. Observation was carried out for a 14-day post treatment period. Body weights were recorded prior to administration of the test material, again 7 days post dosing and at study termination (14 days). At termination, all surviving animals were killed and subjected to a gross necropsy examination.

Result : There were no deaths during the study.
The only clinical observation with the exception of skin irritation was soft stool in all animals. This was observed 3 hours after dosing and returned to normal by day 2. Skin irritation was observed in all animals and ranged from slight to severe for erythema and edema, from slight to marked for atonia, desquamation and fissuring and from slight to moderate for coriaceousness. Other dermal irritation seen included blanching and subcutaneous hemorrhage.
All animals had gained weight by the end of the study. At necropsy, except for the skin lesions no other visible lesions were recorded.

Reliability : (1) valid without restriction

(12)

Type : LD_{50}
Species : Rabbit
Test substance : Various Base oils

Remark : CONCAWE summarized the data available on the acute dermal toxicity of lubricating oil base stocks in rabbits. The data are shown in the following table.

	CAS No	Dermal LD_{50} (g/kg)	API Report No.
Paraffinic distillates	.		
Solvent dewaxed, light			
API 78-9	64742-56-9	>5	29-33104
Solvent dewaxed, heavy			
API 78-10*	64742-56-0	>5	29-33105
API 79-3	64742-65-0	>5	29-33067
API 79-4	64742-65-0	>5	29-33066
API 79-5	64742-65-0	>5	29-33068
Naphthenic distillates			
Solvent refined, light			
API 78-5	64741-97-5	>5	29-33106
Solvent refined, heavy			
API 79-1	64741-96-4	>5	29-33065
Hydrotreated, heavy			
API 83-15	64742-52-5	>2	33-32639

* Although this material is not included in the HPV Lubricating base stocks category, it is similar to other materials in the category and provides supportive information.

(2) (3) (4) (5) (6) (7) (8) (14) (81)

5.2.1 SKIN IRRITATION

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : None - undiluted
PDII : 4.3
Result : Moderately irritating
Method : Draize Test
Year : 1986
GLP : Yes
Test substance : Unrefined base oil Sample API 84-01 [CAS 64741-50-0] See section 1.1.1.

Method : 0.5 ml of undiluted test material was applied to the shorn dorsal skin in two areas on each of 6 male rabbits. One area was intact and the other abraded skin. The treated area was then covered with an occlusive dressing. After 24 hours, the dressing was removed and the treated skin was wiped to remove any residue of test material. The degree of erythema and edema was recorded according to the Draize scale. A second reading of skin responses was made at 72 hours and again at 96 hours, 7 and 14 days. Results of the 24 and 72-hour readings were used to determine the Primary Irritation Index.

Result : One animal died on day 10 even though there had been no signs of ill health previously. Irritation scores given below are averages from 5 animals.

Observation period	Erythema Intact	Erythema Abraded	Edema Intact	Edema Abraded	Average Score
24 hrs.	2.3	2.5	2.3	2.3	4.8
72 hrs.	1.8	2.0	1.7	2.0	3.8
96 hrs.	1.5	1.7	1.0	1.0	2.6
7 days	0.3	0.3	0.3	0.5	0.8
14 days	0	0	0	0	0

Primary dermal irritation index: 4.3

Reliability : (1) valid without restriction

(13)

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : None - undiluted
PDII : 5.4
Result : Moderately irritating
Method : Draize Test
Year : 1986
GLP : Yes
Test substance : Highly refined Base oil Sample API 83-12 [CAS64742-53-6] See section 1.1.1.

Method : 0.5 ml of undiluted test material was applied to the shorn skin in two areas on each of 6 male rabbits. One area was intact and the other abraded skin. The treated area was then covered with an occlusive dressing. After 24 hours, the dressing was removed and the treated was wiped to remove any residue of test material. The degree of erythema and edema was recorded according to the Draize scale. A second reading of skin responses was made at 72 hours and again at 96 hours, 7 and 14 days. Results of the 24 and 72-hour readings were used to determine the Primary Irritation Index.

Result : Average Irritation scores are given below:

Observation period	Erythema Intact	Erythema Abraded	Edema Intact	Edema Abraded	Average Score
24 hrs.	2.3	2.3	2.7	2.7	5.0
72 hrs.	3.0	3.0	2.5	3.0	5.8
96 hrs.	2.7	2.8	2.7	3.0	5.6
7 days	1.3	2.2	0.8	1.7	3.0
14 days	0	0	0	0	0

Primary dermal irritation index: 5.4

Reliability : (1) valid without restriction

(12)

Species : Rabbit
Concentration : Undiluted
Exposure time : 24 hour(s)
Test substance : Various base oils

Remark : CONCAWE summarized the data available on skin irritation for the lubricating oil base stocks. The data are shown in the following table.

	Irritation*	API Report
Paraffinic distillates		
Solvent dewaxed, light		
API 78-9 (64742-56-9)	Slight (0.6)	29-33104
Solvent dewaxed, heavy		
API 78-10*** (64742-56-0)	Non (0.27)	29-33105
API 79-3 (64742-65-0)	Non (0.33)	29-33067
API 79-4 (64742-65-0)	Non (0.34)	29-33066
API 79-5 (64742-65-0)	Non (0.38)	29-33068
White mineral oil***	Slight	Hoekstra & Phillips
Naphthenic distillates		
Solvent refined, light		
API 78-5 (64741-97-5)	Slight (0.65)	29-33106
Solvent refined, heavy		
API 79-1 (64741-96-4)	Slight (0.8)	29-33065
Hydrotreated, heavy		
API 83-15 (64742-52-5)	Slight (1.3)**	33-32639

* NB Irritation described as slight, moderate or non-irritating in the original reports (Mean irritation score given in parentheses)
** Irritation index
*** Although these materials are not included in the HPV Lubricating base stocks category, they are similar to other materials in the category and provide supportive information.

(2) (3) (4) (5) (6) (7) (8) (14) (81)

5.2.2 EYE IRRITATION

Species : Rabbit
Concentration : Undiluted
Dose : 0.1 ml
Number of animals : 9
Method : Draize Test
Year : 1986
GLP : Yes
Test substance : Unrefined base oil Sample API 84-01 [CAS 64741-50-0] See section 1.1.1.

Method : 0.1 ml of undiluted test material was applied to the corneal surface of one eye of each of 9 rabbits, the other eye was untreated and served as control. After 20 to 30 seconds, the treated eyes of 3 rabbits were washed with lukewarm water for 1 minute. Eyes of the other 6 rabbits were not washed.
Readings of ocular lesions for all animals were made at 1, 24, 48, 72 hours and 7 days after treatment. Sodium fluorescein was used to aid in revealing possible corneal injury.

Result : One animal died on day 7 but this was not considered to be treatment related. The test material did not cause a pain response, corneal or iridial irritation. The eye irritation that occurred had cleared by 48 hours.
The primary eye irritation scores (according to the standard Draize scoring procedure) were as follows:

Period	Unwashed eyes	Washed eyes
1 hour	3.0	4.0
24 hours	1.7	0

Scores of 0 were recorded at all other observation times.

Reliability : (1) valid without restriction

(13)

Species : Rabbit
Concentration : Undiluted
Dose : 0.1 ml
Number of animals : 9
Method : Draize Test
Year : 1986
GLP : Yes
Test substance : Highly refined Base oil Sample API 83-12 [CAS64742-53-6] See section 1.1.1.

Method : 0.1 ml of undiluted test material was applied to the corneal surface of one eye of each of 9 rabbits, the other eye was untreated and served as control. After 20 to 30 seconds, the treated eyes of 3 rabbits were washed with lukewarm water for 1 minute. Eyes of the other 6 rabbits were not washed. Readings of ocular lesions for all animals were made at 1, 24, 48, 72 hours and 7 days after treatment. Sodium fluorescein was used to aid in revealing possible corneal injury.

Result : There was no pain response during instillation of the test material and no corneal or iridial irritation was seen during the study. Any irritation that occurred had cleared by 48 hours. The primary eye irritation scores for the first 48 hours of the study were as follows:

Period	Unwashed eyes	Washed eyes
1 hour	2.7	2.0
24 hours	0.3	0
48 hours	0	0

Reliability : (1) valid without restriction

(12)

Species : Rabbit
Concentration : Undiluted
Dose : 0.1 ml
Test substance : Various base oils

Remark : CONCAWE summarized the data available on eye irritation for following table.

	Irritation*	API report No.
Paraffinic distillates		
Solvent dewaxed, light		
API 78-9 (64742-56-9)	Slight	29-33104
Solvent dewaxed, heavy		
API 78-10** (64742=56-0)	Non	29-33105
API 79-3 (64742-65-0)	Non	29-33067
API 79-4 (64742-65-0)	Non	29-33066
API 79-5 (64742-65-0)	Non	29-33068
Naphthenic distillates		
Solvent refined, light		
API 78-5 (64741-97-5)	Non	29-33106
Solvent refined, heavy		
API 79-1 (64741-96-4)	Non	29-33065
Hydrotreated, heavy		
API 83-15 (64742-52-5)	Slight	33-32639

Other mineral oils

Paraffin oil**	Slight	Carpenter & Smyth

* Irritation described as slight, moderate or non-irritating

** Although these materials are not included in the HPV Lubricating base stocks category, they are similar to other materials in the category and provide supportive information.

(2) (3) (4) (5) (6) (7) (8) (14) (77) (81)

5.3 SENSITIZATION

Type	:	Buehler Test
Species	:	Guinea pig
Concentration	:	1st: Induction 25 % occlusive epicutaneous 2nd: Challenge 1 % occlusive epicutaneous
Number of animals	:	10
Vehicle	:	Paraffin oil
Result	:	Not sensitizing
Year	:	1986
GLP	:	Yes
Test substance	:	Unrefined base other TS: Unrefined base oil Sample API 84-01 [CAS 64741-50-0] See section 1.1.1.
Method	:	0.4 ml of a 25% mixture of test material and paraffin oil was applied under an occlusive dressing to the shorn skin of 10 male and 10 female animals. 6 hours after application the dressings were removed and the skin wiped to remove residues of test material. The animals received one application each weeks. The same application site was used each time. 2 weeks following the third application, a challenge dose (0.4 ml of a 1% mixture in paraffin oil) was applied in the same manner as the sensitizing doses. A previously untreated site was used for the challenge application. The application sites for sensitizing and challenge doses were read for erythema and edema 24 and 48 hours after patch removal. To assist in the reading of the response to the final challenge dose the test site was depilated 3 hours prior to reading by using a commercially available depilatory cream. Positive control (2,4-dinitrochlorobenzene at 0.3% in 80% aqueous ethanol), vehicle control and naive control groups were included in this study and the procedure for these was the same as for the test groups.
Result	:	The criteria used to evaluate the responses are described in the report as follows: Determination of sensitization was based upon reactions to the challenge dose. Grades of 1 or greater in the test animals indicate evidence of sensitization, provided grades of less than 1 are seen in the naive controls. If grades of 1 or greater are noted in the naive control animals, then the reactions of test animals that exceed the most severe naive control reaction are considered sensitization reactions. Using these criteria, none of the test animals became sensitized following treatment with API 84-01. In contrast, all the positive control animals were sensitized by their treatment.
Reliability	:	(1) valid without restriction

(13)

Type	:	Buehler Test
Species	:	Guinea pig
Concentration	:	1st: Induction 50 % occlusive epicutaneous 2nd: Challenge 1 % occlusive epicutaneous
Number of animals	:	10
Vehicle	:	Paraffin oil
Result	:	Not sensitizing
Year	:	1986
GLP	:	Yes
Test substance	:	Highly refined Base oil Sample API 83-12 [CAS64742-53-6] See section 1.1.1.

Method : 0.4 ml of a 50% mixture of test material and paraffin oil was applied under an occlusive dressing to the shorn skin of 10 male and 10 female animals. 6 hours after application, the dressings were removed and the skin wiped to remove residues of test material. The animals received one application each week for 3 weeks. The same application site was used each time. 2 weeks following the third application, a challenge dose (0.4 ml of a 1% mixture in paraffin oil) was applied in the same manner as the sensitizing doses. A previously untreated site was used for the challenge application. The application sites for sensitizing and challenge doses were read for erythema and edema 24 and 48 hours after patch removal. To assist in the reading of the response to the final challenge dose the test site was depilated 3 hours prior to reading by using a commercially available depilatory cream.

Positive control (2,4-dinitrochlorobenzene at 0.3% in 80% aqueous ethanol), vehicle control and naive control groups were included in this study and the procedure for these was the same as for the test groups.

Result : The criteria used to evaluate the responses are described in the report as follows:
Determination of sensitization was based upon reactions to the challenge dose. Grades of 1 or greater in the test animals indicate evidence of sensitization, provided grades of less than 1 are seen in the naive controls. If grades of 1 or greater are noted in the naive control animals, then the reactions of test animals that exceed the most severe naive control reaction are considered sensitization reactions.

One animal had a score of 0.5 after challenge with API 83-12. In contrast, all the positive control animals were sensitized by their treatment. The sample of API 83-12 was therefore non sensitizing.

Reliability : (1) valid without restriction

(12)

Type	:	Buehler Test
Species	:	Guinea pig
Test substance	:	Various base oils

Remark : CONCAWE summarized the data available on skin sensitization for the lubricating oil basestocks. The methods and criteria used were the same as those described in the previous two robust summaries. The data are shown in the following table.

		Sensitization	API Report
Paraffinic distillates			
Solvent dewaxed, light			
API 78-9	64742-56-9	Non	29-33104
Solvent dewaxed, heavy			
API 78-10*	64742-56-0	Non	29-33105
API 79-3	64742-65-0	Non	29-33067
API 79-4	64742-65-0	Non	29-33066
API 79-5	64742-65-0	Non	29-33068
Naphthenic distillates			
Solvent refined, light			
API 78-5	64741-97-5	Non	29-33106
Solvent refined, heavy			
API 79-1	64741-96-4	Non	29-33065
Hydrotreated, heavy			
API 83-15	64742-52-5	Non	33-32639

* Although this material is not included in the HPV Lubricating base stocks category, it is similar to other materials in the category and provides supportive information.

(2) (3) (4) (5) (6) (7) (8) (14) (81)

5.4 REPEATED DOSE TOXICITY

Type : Sub-acute
Species : Rat
Sex : Male/female
Strain : No data
Route of admin. : Inhalation
Exposure period : 14 days
Frequency of treatm. : Six hours per day
Control group : Yes
NOAEL : > 50 mg/m³
Year : 1989
GLP : No data
Test substance : Two samples of highly refined, solvent extracted dewaxed paraffinic base oil

Method : Groups of 5 male and 5 female rats were exposed to oil mists generated from two highly refined oils. Exposures were by inhalation six hours each day for a total of 10 days. The two oils were examined in separate experiments. The dose groups were:

Group	Mean actual concentration (mg/m^3)	Mass median particle size (µm)
Controls	Air only	N/A
Oil 1	55	1.5
	507	1.9
	1507	2.2
Oil 2	Air only	N/A
	50	1.5
	513	1.9
	1480	2.2

No further experimental details are provided.

Remark : A further two week inhalation study in rats has been reported for two mineral oil mists (Skyberg et al, 1990). The results largely confirm those described by Whitman et al. with respect to liver weight changes and histological observations in respiratory tissues.

Result : Oil 1
All treated animals survived to study termination.
The fur of all animals was saturated with test material and the amount of material present was clearly related to the exposure concentration.
Alopecia and scabs subsequently formed in the highest 2 dose groups.
Animals in the highest dose group were relatively unresponsive to auditory stimulation. Decreased body weight associated with a decrease in food consumption was recorded for the high dose animals.

Biologically significant increases in relative lung and liver weights were observed in the males and females in the high dose group but only in the mid dose females.
An increase in white cell counts and the percentage of neutrophils and a decrease in the percentage lymphocytes was observed in the high dose groups only. There were no treatment related histopathological changes in the lowest 2 dose groups. Animals in the highest dose group exhibited the same changes as those observed in the nasoturbinates and lungs of animals exposed to oil 2 (See below)

Oil 2
Clinical observations were the same as for those animals exposed to Oil 1, except that there was no scabbing and no treatment related alterations in food consumption. There was a biologically significant increase in absolute and relative lung weights in males and females at the high dose and in females only at the mid dose.
Apart from elevated liver alanine and aspartate transaminase levels in the high dose females there were no other treatment related effects.
Histological effects considered to be treatment related consisted of an increase in the amount of perivascular and peribronchial lymphoid proliferations and an increase in mixed inflammatory cell infiltrations in the terminal bronchioles and alveolar ducts of the highest two dose groups.
Increases in the appearance of focal hyperplasia and squamous cell metaplasia of the anterior nasal mucosa associated with inflammatory cell infiltration was observed in the two highest dose groups. These changes were indicative of mild irritation of the nasal mucosa.

The NOELs for the two oils were >50 mg/m^3

Reliability : (4) not assignable
The information is taken from a poster presentation and a reliability score cannot be assigned. However, the data are supportive of the other study on inhalation of oil mist reported by Dalbey et al.

(120) (126)

Type : Sub-acute
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : 4 weeks
Frequency of treatm. : 6 hours/day, 5 days/week
Doses : 50, 220 & 1000 mg/m^3
Control group : Yes, concurrent no treatment

Year : 1991
GLP : No data
Test substance : 3 base oils

Three materials were examined in this study. The properties of the materials designated SRO, WTO and HBO are shown in the following table.

SRO Solvent refined oil CAS # 64742-70-7
WTO White oil CAS # 8042-47-5. [Prepared by severely hydrotreating a dewaxed feedstock and then acid washing with fuming sulfuric acid.]

HBO Hydrotreated base oil CAS #64742-54-7 [Severely hydrotreated heavy paraffinic oil produced by treatment of the vacuum distillate with hydrogen at high temperature and pressure (hydrotreating and hydrocracking)].

	SRO	WTO	HBO
Viscosity at 100 °F	106	85	161
Pour point (°F)	20	15	-5
API Gravity	32.8	34.6	33.6
Furfural (ppm)	1	0	<1
Nitrogen (ppm)	44	-	8
Sulfur (wt.%)	0.20	-	<0.06
Composition (wt.%)			
Paraffins	36	60	29.7
Mononaphthenes	22.3	-	30.6
Polynaphthenes	22.3	-	37.3
Monoaromatics	12.8	0	0.6
Diaromatics	3.3	0	0.8
Polyaromatics	1.4	0	1.0
Unidentified aromatics	0.4	0	0
Aromatic sulfur types	1.1	0	0

Method : Groups of 10 male and 10 female rats, 11-12 weeks of age, were exposed to aerosol concentrations of the three test materials at nominal concentrations of 0, 50, 220 and 1000 mg/m³.
Exposures were for 6 hours each day, 5 days each week for 4 weeks.
Total number of exposures for each of the three test materials was: 17, 18 and 20 days for SRO, WTO and HBO respectively. Food and water were available ad libitum during non-exposure periods. Clinical observations were made prior to each exposure and body weights were recorded weekly.
Animals were sacrificed within 72 hours of the last exposure after being fasted overnight. Blood samples were taken for a range of hematology and serum chemical parameters. The hematological parameters consisted of: Total white and red cells, hemoglobin, hematocrit, MCV, MCH, and MCHC. A differential white cell count was also conducted.
The following chemical parameters were measured: Alanine transferase, albumin, albumin/globulin ratio, alkaline phosphatase, aspartate aminotransferase, total bilirubin, calcium, chloride, cholesterol, creatinine, globulin, glucose, iron, lactate dehydrogenase, inorganic phosphorus, potassium, total protein, sodium, triglycerides, urea nitrogen and uric acid.
All animals were necropsied and the following organs were weighed: gonads, heart, kidneys, liver, spleen, and thymus.
The right middle lobe of the lung was weighed immediately after removal and again after drying.

H&E sections were prepared and examined of the following tissues from all control and high dose group animals: heart, kidney, liver, lung, four locations in the nasal turbinates, spleen, gonads, thymus and tracheobronchial lymph nodes.
Sperm from the cauda epididymis of each control and high dose male was examined for an assessment of sperm morphology.

Statistical analysis
Data were analyzed by one-way analysis of vatriance.
A probability of Type I error of <5% (P<0.05) was considered to be statistically significant. Comparison of means was performed by Duncan's multiple range test or the Student-Neuman-Keuls multiple comparison.
Data obtained from exposure to a given test article were analyzed together. No statistical procedures were carried out to compare the effects of different test articles with each other.

Result :

Chamber concentrations
The aerosol concentrations were comparable among the three base stocks.
Qualitatively, the aerosols were virtually identical to each liquid base oil.
The actual concentrations for each of the aerosols was as

	Nominal	Actual
SRO	0	0
	50	50 ±10
	220	210 ±10
	1000	1020 ±60
WTO	0	0
	50	50 ±10
	220	210 ±10
	1000	980 ±20
HBO	0	0
	50	47 ±2
	220	220 ±10
	1000	980 ±50

The mass median diameter was well under 2µm for each base stock

Toxicity assessment
Apart from occasional loose stool there were no treatment related clinical observations and body weights were unaffected by exposure.
No treatment related effects were found in any of the hematological or clinical chemical parameters that were measured.
The percent sperm with aberrant morphology, including breakage, was unaffected by exposure to any of the three base oils.
There were no treatment-related observations at necropsy and, with the exception of the lungs, there were no significant changes in organ weights .
Wet and dry lung weights increased in a dose-related manner. The percentage increases in wet weight are shown in the following table. For simplicity increases are shown to nearest whole numbers

		% Increase in wet lung weight		
Sex	**Dose (mg/m^3)**	**SRO**	**WTO**	**HBO**
Female				
	50	3	8	2
	210	4	23*	34*
	1000	38*	64*	36*

Male

50	5	-	1
210	12*	1	6
1000	33*	31*	32*

* denotes differences that are statistically significant (P<0.05) compared to controls.

The ratios of wet to dry lung weights were significantly increased for both sexes at the highest dose concentration for all three base oils.

Morphologically, treatment related changes were only observed in the lungs and tracheobronchial lymph nodes.

Foamy macrophages with numerous vacuoles of varying size were present in the alveolar spaces of the lungs of many of the exposed animals. The histological changes are summarized in the following table.

No. of animals in each group with a given histopathological change

Tissue/change	Dose group 50	210	1000
SRO			
Lung			
1-2 Foamy macrophages (FM)	20	20	20
3-6 FM	0	0	20
Thickened alveolar wall	0	0	0
FM in alveolar interstitium	0	0	0
Mild alveolar PMN infiltrate	0	5	20
Lymph nodes			
Anterior mediastinal			
Macrophage accumulation	NE	NE	9
Tracheobronchial			
FM accumulation	NE	NE	19
Macrophage accumulation	NE	NE	0
WTO			
Lung			
1-2 Foamy macrophages (FM)	20	20	20
3-6 FM	0	0	20
Thickened alveolar wall	0	0	0
FM in alveolar interstitium	0	0	0
Mild alveolar PMN infiltrate	0	0	19
Lymph nodes			
Anterior mediastinal			
Macrophage accumulation	NE	NE	0
Tracheobronchial			
FM accumulation	NE	NE	0
Macrophage accumulation	NE	NE	19
HBO			
Lung			
1-2 Foamy macrophages (FM)	0	16	16
3-6 FM	0	0	16
Thickened alveolar wall	0	0	16
FM in alveolar interstitium	0	0	16
Mild alveolar PMN infiltrate	0	0	0
Lymph nodes			
Anterior mediastinal			
Macrophage accumulation	NE	NE	2
Tracheobronchial			

FM accumulation	NE	NE	0
Macrophage accumulation	NE	NE	3

NE denotes Not Evaluated
Only 16 animals in the HBO high dose group wereexamined

In conclusion the NOAELs and LOAELs for the oils can be summarized thus:

Oil	LOAEL (mg/m^3)	NOAEL (mg/m^3)
SRO	210	50
WTO	210	50
HBO	210	50

Reliability : (2) valid with restrictions
It is not clear whether the study was carried out according to GLP, but otherwise it was a well conducted and well reported study.

(83)

Species : Rabbit
Sex : Male/female
Strain : New Zealand white
Route of admin. : Dermal
Exposure period : 6 hours each day
Frequency of treatm. : 3 times each week for a total of 12 applications
Doses : 200, 1000 and 2000 mg/kg
Control group : Yes
Year : 1986
GLP : Yes
Test substance : Unrefined base oil Sample API 84-01 [CAS 64741-50-0] See section 1.1.1.

Method : Undiluted API 84-01 was applied at doses of 200, 1000 and 2000 mg/kg/day to the shorn dorsal skin of groups of five male and five female rabbits. The test material was applied to the skin 3 times each week for 4 weeks (12 applications total). The applied material was covered with an occlusive dressing for 6 hours, which was then removed and the skin was wiped with a dry gauze to remove any residual material. A group of five rabbits of each sex served as sham controls.
The test skin site of each animal was examined and scored for irritation prior to each application of test material. Mortality and moribundity checks were performed twice daily and body weights were recorded weekly. At termination, blood samples were taken for a range of hematological and clinical chemical measurements. Urine samples were also collected and frozen for possible future examination. A complete gross necropsy was performed on all animals. Major organs were weighed and tissues were processed for subsequent histopathological examination.

Result : Three animals died during the study but these were not dose-related and were, therefore, considered unrelated to treatment. Sporadic clinical signs were also unrelated to treatment.
In the high dose group, body weight gains were affected by treatment. In the females, there was a group net loss in weight whereas in the males the gains were significantly less than controls. These effects were largely due to effects on growth rate during the first week of the study. A mean irritation index was calculated for each group each day and also for each treatment group overall. The value was determined from Draize scores for erythema and edema for each animal. The mean irritation scores for each

group were:

Group	Irritation score
Control (male)	0
Control (female)	0
200 mg/kg (male)	0.5
200 mg/kg (female)	0.4
1000 mg/kg (male)	1.7
1000 mg/kg (female)	2.0
2000 mg/kg (male)	3.1
2000 mg/kg (female)	3.2

There were no statistical differences between treated and control groups for any of the hematological determinations. These were: Total red blood cells, total white blood cells, hemoglobin concentration and hematocrit %.

The clinical chemical data for the treated and control males was similar. In the females, there was a reduced BUN and an increased SGPT for the low dose females. Since no other differences were noted and that values were within normal limits the effects were not considered to be toxicologically significant. The clinical chemical measurements consisted of: glucose, BUN, SGOT, SGPT, ALP and total protein.

The following absolute and relative organ weight differences (compared to controls) were recorded.

	Males	Females
2000 mg/kg		
Relative liver wt.	Increased	Increased
Relative kidney wt.	Increased	Increased
Relative pituitary wt.	Increased	
Relative left testis wt.	Decreased	
Relative brain wt.		Increased
1000 mg/kg		
Abs. Rt. kidneywt.	Decreased	
Abs. Heart wt.		Decreased

None of the organ weight differences were considered treatment-related. The higher than control relative organ weights were considered as a function of the reduced body weights in the affected animals.

The only findings at gross necropsy were confined to the treated skin. These consisted of dry, scaly, rough, and/or reddened skin and thickened dermis. These findings were noted throughout the treatment groups. There were no treatment-related gross necropsy findings in the internal organs.

Microscopic pathology findings were also largely confined to the skin. Slight to moderate proliferative changes of the skin were present in all of the male and female rabbits in the highest dose group.

The testes of one of the five males in the high dose group had bilateral diffuse tubular hypoplasia accompanied by aspermatogenesis and hypoplasia of the epididymis. These changes were considered to represent immature testes. Similar changes were not seen in the other

animals in this dose group.

Reliability : (1) valid without restriction

(11)

Species : Rabbit
Sex : Male/female
Strain : New Zealand white
Route of admin. : Dermal
Exposure period : 6 hours each day
Frequency of treatm. : 3 times each week for a total of 12 applications
Doses : 200, 1000 and 2000 mg/kg
Control group : Yes
Year : 1986
GLP : Yes
Test substance : Highly refined Base oil Sample API 83-12 [CAS64742-53-6] See section 1.1.1.

Method : Undiluted API 83-12 was applied at doses of 200, 1000 and 2000 mg/kg/day to the shorn dorsal skin of groups of five male and five female rabbits. The test material was applied to the skin 3 times each week for 4 weeks (12 applications total). The applied material was covered with an occlusive dressing for 6 hours, which was then removed and the skin was wiped with a dry gauze to remove any residual material. A group of five rabbits of each sex served as sham controls.
The test skin site of each animal was examined and scored for irritation prior to each application of test material. Mortality and moribundity checks were performed twice daily and body weights were recorded weekly.
At termination, blood samples were taken for a range of hematological and clinical chemical measurements. Urine samples were also collected and frozen for possible future examination.
A complete gross necropsy was performed on all animals. Major organs were weighed and tissues were processed for subsequent histopathological examination.

Result : No deaths occurred during the study.
Skin irritation occurred to varying degrees in all animals treated with API 83-12. There was moderate irritation in the high dose males and females. In the mid dose group moderate irritation occurred in the females and slight irritation in the males. In the low dose group minimal irritation occurred in both sexes. The overall mean irritation scores were:

Dose level (mg/kg)	Males	Females
Control 0	0	0
200	0.1	0.4
1000	2.0	2.2
2000	2.6	3.1

Soft stool was also observed in several animals but this also occurred in a control male was not considered to be dose related. All high dose females appeared thin and this was considered to be treatment related.
Body weight gains were reduced in the high dose males and females and in the mid dose females when compared to their respective controls.
Overall weight changes (kg) are shown in the following table

Dose level (mg/kg)	Males	Females
Control 0	+0.5	+0.3
200	+0.3	+0.4
1000	+0.3	0.0*
2000	+0.1*	-0.2*

* statistically significant ($p \leq 0.05$)

Clinical chemical and hematological values were considered to be unaffected by treatment. A low value (cf control) for white cell count in the low dose female group was considered incidental since the value was within a normal range and was not a dose-related effect.

Although there were some organ weight differences, they were considered incidental to treatment. The exception was for the absolute testis weights, which were lower in the high dose males and the relative weights of the right testis which were also lower than controls.

At gross necropsy, findings for the skin consisted of dry, scaly, rough, fissured, crusted and/or thickened skin. This was a common finding in all treatment groups.

Histopathological examination revealed slight to moderate proliferative changes in the skin in all rabbits in the high dose group. These changes were accompanied by an increased granulopoeisis of the bone marrow. The testes of 3 of the 5 males in the high dose group had bilateral diffuse tubular hypoplasia accompanied by aspermatogenesis changes observed in either the testes or epididymes of the male rabbits in the mid or low dose groups.
No other treatment-related histopathological changes were recorded.

Reliability : (1) valid without restriction

(10)

Species : Rabbit
Route of admin. : Dermal
Test substance : Various Base oils

Remark : Data on repeated dose dermal studies in rabbits have been summarized elsewhere (CONCAWE 1997).
The attached tabulated summary of information is taken from the CONCAWE publication.

Attached document : Attachment 2: Summary of dermal repeat dose studies.doc

(2) (3) (4) (5) (6) (7) (8) (15) (81) (123)

Species : Rat
Sex : Male/female
Strain : Fischer 344
Route of admin. : Oral feed
Exposure period : 90 days
Frequency of treatm. : Continuous in food
Doses : 0.002, 0.02, 0.2 & 2.0% in the diet
Control group : Yes
Method : OECD Guide-line 408 "Subchronic Oral Toxicity - Rodent: 90-day Study"
Year : 1992
GLP : Yes

Test substance : Six White oils
Six white oils examined in this study were characterized.
Only the average molecular weight and viscosity at 100 °C are shown below:

Sample	Viscosity (cSt)	Average Molecular Weight
N10(A)	3.08	320
N15(H)	3.45	330
P15(H)	3.52	350
N70(A)	7.88	410
N70(H)	7.65	420
P100(H)	11	510

Method : Three related, but separate studies were carried out at the same time on 6 different food grade white oils and 3 food grade waxes.
Only the information on the oils is included here. The information on waxes is included in the Waxes and Related Materials HPV Test Plan.

In the main study, groups of 20 male and 20 female rats were fed diets containing one of 6 different white oils at dietary concentrations of 0.002, 0.02, 0.2 and 2.0% for 90 days. Further groups of 60 male and 60 females were fed untreated control diet. Additionally groups of 20 rats of each sex were fed diets containing 2.0% coconut oil.

The second study was a reversibility study. Groups of 10 rats of each sex were fed diets for 90 days containing one of the 6 different oils at the 2.0% level or coconut oil at 2%. These animals were then fed control diet for 28 days following the 90-days treatment. Groups of 30 rats of each sex served as controls for this reversibility study.

A third study was designed to determine tissue levels of hydrocarbons. In this study, 5 rats of each sex were fed diets containing one of the 6 oils or coconut oil at the 2.0% dietary level for 90 days. Extra groups of rats (5 of each sex) were fed control diet or coconut oil or one of the six oils for 90 days followed by exposure to control diet only for a further 28 days.

In all three studies, animals were monitored for weight, food intakes and clinical condition throughout. An ophthalmic examination was performed prior to treatment and prior to necropsy on the animals in the main study and those for the study of reversibility.
A full necropsy was performed on the main and reversibility were measured on blood samples taken from the animals. Clinical chemical measurements were also made on serum separated from the blood samples. A selection of organs was weighed and a range of tissues retained for subsequent histopathological examination. All tissues from the high dose group and control groups were examined by light intermediate dose groups.
Mineral hydrocarbon levels were measured in a limited number determinations.

Remark : While only one report (three studies) is described here, numerous repeat dose studies on white oils destined for use in foods have been conducted and reported in the open literature.

Recent studies with a low molecular weight white oil have demonstrated

that the F 344 rat is more sensitive in its response to mineral hydrocarbons than the Sprague Dawley rat (Firriolo et al). Indeed other studies on white oils with Sprague Dawley rats (McKee et al) and beagle dogs (Bird et al) have also not resulted in any reported effects .

Result : The six oils tested had average molecular weights ranging from 320 to 510. The effects observed in the study were inversely related to the oil's molecular weight. Thus the oil with the lowest molecular weight caused the most severe effects and at lower dose levels than the higher molecular weight materials. For simplicity, only the results of the highest and lowest molecular weight oils are summarized below. Furthermore, the results of the reversibility study are not given in detail here.
In general, there was evidence of reversibility of the effects but reversibility was not complete for all of the parameters measured.

P 100 H (Average molecular weight 510)
There were no treatment-related clinical signs, nor was there an effect on body weight. Food consumption was increased in the males of the highest dose group but this was less than 10% greater than for the controls. Ophthalmic examination did not reveal any effects. Organ weights, hematology and clinical chemistry were unaffected except for a 10% increase in ASAT in the males in the highest dose group.
There were no treatment-related findings at necropsy and the histological examination did not reveal any treatment-related effects.
A small amount of mineral hydrocarbon was found in the livers of the male rats in the highest dose group.

N 10 A (Average molecular weight 320)
There were no treatment-related clinical signs, nor was there an effect on body weight. Food consumption was increased in the males of the highest dose group but this was less than 10% greater than for the controls. Ophthalmic examination did not reveal any effects.

Organ weights
Increases in organ weights are as shown below, other organ weights were unaffected.

Organ		**Increases (%) at Dietary concentration**			
		Males		**Females**	
		0.2%	**2.0%**	**0.2%**	**2.0%**
Kidney	(abs.)	4	6		5
	(rel.)		7		7
Liver	(abs)	8	11	6	21
	(rel.)	6	12	8	23
Spleen	(abs.)				17
	(rel.)		5		19
MLN*	(abs.)		224		220
	(rel.)		224		226

* NB Mesenteric Lymph Node weights only determined for the 2% dose group in the reversal group of animals and not for the main study animals.

Hematology
In the males in the highest dose group there were increasesin Neutrophils (41%), monocytes (28%) and basophils (200%) In the females, changes

occurred in the 2% and 0.2% dose groups. These were as follows:

	Change (% + or -) at dose level 0.2%	2%
RBC	- 2	- 3
Hemoglobin	- 2	- 3
WBC		+ 23
Differential WBC		
Neutrophils		+ 75
Monocytes		+ 51
Eosinophils		+ 38

Clinical chemistry
In the males there was a reduction in Alkaline phosphatase of 8 and 2% in the 2 and 0.2% dose groups respectively.
Changes in clinical chemical parameters in the females were as follows:

	Change (% + or -) at dose level 0.2%	2%
ALKP	- 12	- 13
ASAT		+ 12
Gamma GT		+ 91
A/G ratio		- 8

Histopathology
Liver
Liver lesions comprised mirogranuloma or granuloma, the distinction between being purely related to size. Lesions were classified as microgranuloma if the average diameter was less than 25% of the average hepatic lobule. The histological features of the two were similar and consisted of collections of macrophages, some with necrotic cells surrounded by inflammatory cells and variable fibrosis.

No lesions were observed in the males whereas granulomas were seen in the females in the highest dose group.
In females in the recovery group 28 days after cessation of exposure, the incidence was unchanged but the severity of the lesions had decreased.

Mesenteric Lymph node
The lymph node lesions comprised focal collections of macrophages, often in the cortical region. The macrophages were lightly vacuolated, giving a slightly foamy appearance to their cytoplasm. Some macrophages had a yellowish-brown pigmentation of varied intensity. The focal collections of macrophages were classified as histiocytosis and were scored as minimal, mild, moderate or marked based on size and abundance. The foci of histiocytosis were not homogeneously distributed; they were often restricted to one node or even to part of one node. Histiocytosis was also found in control rats but was generally restricted to isolated foci and was always classified as minimal. Compared to controls, in males histiocytosis increased down to the 0.2% dose group. In the females, histiocytosis was also observed in the 0.02% dose group.
In the reversibility group the severity and incidence was reduced after being fed control diet for 28 days.

Ileum and jejunum
There was a significant increase in vacuolation of the lamina propria in the high dose female group.

In summary, the NOELs and LOELs for the six oils that were tested are as follows.

Oil	LOEL (histiocytosis) Dietary concentration	NOAEL
N10A	0.02%	
N15H	0.002%	
P15H	0.02%	
N70A	0.02%	
N70H	0.02%	
P100H	-	2.0%

Reliability : (1) valid without restriction

(22) (98) (105)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Modified Ames Assay
System of testing : Salmonella typhimurium strain TA98
Metabolic activation : With
Year : 1984
Test substance : Various base oils

Method : The method differed from the standard pre- incubation Ames assay in the following respects.

A DMSO extract of the test materials was tested in the assay.

The S9 fraction was obtained from Araclor-induced hamsters.

An eightfold concentration of S-9 was used in the assays.

Twofold concentration of cofactor NADP was used.

The DMSO extracts were tested over a range of concentrations that permitted the construction of a dose-response curve.

A Mutagenicity Index was determined for each assay. This was the tangent to the dose response curve at zero dose.

An assay was judged to be positive if the Mutagenicity Index was greater than 1.0

Result : Roy describes the mutagenicity results for a range of petroleum-derived materials, 28 of which were lubricating oil base stocks.
A Mutagenicity Index (MI) was determined for each test material and this was compared to the PAC content and to a carcinogenicity index that had also been determined for each material.
The results were as follows.

Sample	MI*	%PAC**	%T***	%T/LP****
5	0.9	0.9	0	4.17
6	0	0.3	0	0
7	0.9	0.9	2	4.17
8	0	0.6	0	0
9	0	0.3	0	0
10	0	0.7	2	3.28
12	2.4	3.1	4	5.93
13	9.1	10	26	71
14	0	0.7	2	3.45
15	0	0.2	0	0
16	3.9	3.7	6	1.6
17	4	3.1	8	14.3
18	3.6	4.9	10	21.7
19	6.5	5.2	10	23.4
20	9.2	7.7	40	138
26	0	0.5	2	2
27	0	0.5	2	3.92
28	0	0.3	0	0
29	0	0.6	0	0
30	0	0.6	0	0
32	10	12	54	154
33	5.9	7.8	42	73.7
34	4.1	4.1	50	104
35	1.2	1.2	4	6.25
36	2.1	1.5	18	38.3
37	0	0.7	2	2.13
38	4.5	4.6	24	46.2
39	0	1.2	0	0

* MI denotes Mutagenicity index.

** %PAC is weight% of 3-7 ring PNAs in the oil.

*** %T is the percentage of mice with tumors in skin carcinogenicity studies reported elsewhere.

**** %T/LP is the percentage of mice with tumors multiplied by the reciprocal of the latency period. The author describes this as a carcinogenic potency index.

Test substance : The baseoils tested had PAC contents ranging from 0.2 to 12%. It is generally recognized that those base oils with PAC contents less than 3% are highly refined oils whereas those with greater values are considered to be poorly refined. This distinction was recognized and used by the EU in its classification of base oils. (EU, 1994; CONCAWE 1994)

Conclusion : Base stocks with no or low concentrations of PACs have low Mutagenicity indices. Also, those oils that were negative in the modified Ames assay (MI < 1.0) were not carcinogenic in mouse skin painting studies.
Those oils which were positive in the modified Ames assay had significant levels of PACs and were carcinogenic.

Reliability : (1) valid without restriction

(24) (26) (111)

Type : Modified Ames Assay
System of testing : Salmonella typhimurium strain TA98
Metabolic activation : With
Result : Negative

GLP : No data
Test substance : Residual base oils

Method : The test substance (Canthus 1000, a deasphalted, dewaxed residual oil) was diluted 1:5 in DMSO and then shaken, centrifuged and separated into 2 fractions. Two assays were conducted for the test substance: an initial assay and a repeat assay. All plates were evaluated following approximately two days of incubation. Test volumes of 5, 10, 15, 20, 30, 40, 50 and 60 µl/plate were prepared by dilution of the DMSO fraction in DMSO and dosed at a final volume of 60 µl. The volumes were added to each plate with metabolic activation (hamster S9) and tester strain TA98 following the procedures outlined by Blackburn et al., (1986) and the methods described in the American Society for Testing Materials (ASTM) document, "The Standard Test Method for Determining Carcinogenic Potential of Virgin Base Oils in Metalworking Fluids". The same test volumes were used in the repeat assay. A positive control and vehicle control were tested concurrently.

Linear regression analysis (ASTM: E 1687-95) was performed on the test substances which caused an increase in the mean number of revertant colonies when compared to the vehicle control. Only data from the linear portion of the dose response curve was used to generate the mutagenicity index (MI). If the increase in revertant colonies was not statistically significant or if there was no increase in the mean umber of revertant colonies, then the MI value was considered to be 0 (revertants/µl DMSO extract).

Data from both the initial and repeat assays on the test material (Canthus 1000) were pooled to generate a single linear MI value. With this procedure, an MI value > 1.0 (revertants/µl DMSO extract) is considered indicative of a potential dermal carcinogen in mice (Blackburn et al, 1996). Conversely, a test substance is considered unlikely to be carcinogenic in mouse skin when the MI value is < 1.0 (revertants/µl DMSO extract).

Result : The MI for Canthus 1000 was determined to be 0.2 revertants/µl DMSO extract.
Thus, under the conditions of this study, Canthus 1000 was considered negative for inducing frameshift mutations in Salmonella typhimurium.

Reliability : (4) not assignable
This summary is based on a summary of the results of a study.
It is not possible, therefore to assign a reliabilty to this study.
The data however are useful, together with other similar data to demonstrate that residual base oils are not mutagenic in a modified Ames assay.

(20) (24) (25) (97)

Type : Modified Ames Assay
System of testing : Salmonella typhimurium strain TA98
Metabolic activation : With
Result : Negative

Remark : Summaries are available on Modified Ames assays that have been carried out on 3 additional residual base oils and a vacuum residuum.
The results and references to the studies are shown below. Under the conditions of this study, the test materials were considered negative for inducing frameshift mutations in Salmonella typhimurium.

Material	Mutagenicity Index (MI)	Reference
Vacuum residuum	0.8	Petrolabs (1998)
Bright stock	0.11	Petrolabs (2000)
150 SUS Bright stock	0	EMBSI
150 Solvent Bright stock	0	EMBSI

Reliability : (4) not assignable
This summary is based on a summary of the results of a study. It is not possible, therefore, to assign a reliability to this study. The data, however, are useful, together with other similar data, to demonstrate that residual base oils are not mutagenic in a modified Ames assay.
(85) (109) (110)

Type : Mouse lymphoma assays - General comments

Remark : The mouse lymphoma assays that have been described have been compromised, either because there was no dose-related response or there was a limitation due to poor solubility of the test material.
For these reasons, no mouse lymphoma assays have been described in detail in this robust summary.

However, the results of these mouse lymphoma assays (with and without metabolic activation) carried out on seven different lubricating base oils are summarized in the following table.

Sample	Result	Reference
Paraffinic base oils		**(API report No.)**
API 78-9	Equivocal No dose response	28-31864
API 78-10	Equivocal No dose response	28-31868
API 79-3	Equivocal No dose response	28-31865
API 79-4	Equivocal No dose response	28-31866
API 79-5	Equivocal No dose response	28-31867
Naphthenic base oils		
API 78-5	Negative [(a)]	28-32359
API 79-1	Negative [(a)]	29-32360

[(a)] Solubility limited the evaluation

Reliability : (1) valid without restriction

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley

Route of admin. : Gavage
Exposure period : 5 days
Doses : 0.5, 1.67 & 5.0 g/kg/day
Result : Negative
Year : 1982
GLP : No
Test substance : Highly refined base oil

Method : Test material was administered orally to groups of five male and five female rats daily for 5 days. The dose groups were 0.5, 1.67 and 5.0 g/kg/day. Additionally five animals of each sex were orally dosed with 0.9% saline daily for five days; these animals served a negative controls. Positive controls consisted of ten males and ten females that were given a single i.p. dose of triethylenmelamine (TEM) in saline at a dose level of 1.0 mg TEM /kg. This positive control substance was administered six hours before termination of the study.

Three hours prior to kill, the animals were injected i.p. with 4 mg/kg of colchicine to arrest cell division.
The animals were killed with CO_2 and the adhering tissue and epiphyses of both tibiae were removed. The marrow was removed and transferred to Hank's balanced salt solution. The marrow button was collected by centrifugation and resuspended in 0.075M KCl. The centrifugation was repeated and the pellet resuspended in fixative (methanol:acetic acid, 3:1). The fixative was changed once and the cells left overnight at 4 °C.
Cells in fixative were dropped onto glass slides which were air-dried and stained with Giemsa.

Each slide was scored for chromosomal aberrations. Scoring was for gaps, breaks, fragments and reunion figures. Routinely 50 spreads were read for each animal. A mitotic index based on at least 500 cells was recorded. This index was calculated by scoring the number of cells in mitosis per 500 cells on each slide read.

Result : The results of the assay are summarized in the following table.

Group size	Frequency of aberrations Str*	Num**	% cells 1+ aberrations	2+ aberrations	Mitotic index
-ve control (saline)					
10 (male)	0.002	0.018	0.2	0	3.8
9 (female)	>0.007	0.047	0.7	0.2	2.6
+ve control (TEM @1.0 mg/kg)					
10 (male)	>0.791*	0.032	21.7**	12.8**	2.6
9 (female)	>1.211**	0.048	26.6**	18.8**	1.6
API 78-5 (0.5 g/kg/day)					
8 (male)	0.008	0.030	0.5	0.3	2.4
9 (female)	0.004	0.011ss	0.4	0	4.0
API 78-5 (1.67 g/kg/day)					
9 (male)	0.004	0.018	0.4	0	2.0
10 (female)	>0.008	0.028	0.8	0.2	4.3
API (5 g/kg/day)					
10 (male)	0.002	0.008	0.2	0	6.9
10 (female)	0.006	0.006ss	0.6	0	4.5

It was concluded that the aberration frequency of groups treated with test material did not differ from that of the negative controls at any tested dose.

Furthermore, the there was no increase in the percentage of cells showing one or more structural or numerical aberrations. Therefore it was concluded that the test material was negative in this assay.

Reliability : (1) valid without restriction
Although there is no statement relating to GLP compliance, the study was subjected to a QA audit.

(9)

Type : Cytogenetic assay
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Gavage
Exposure period : 5 days
Result : Negative
GLP : No
Test substance : Lubricating base oils (various)

Remark : Conaway et al published a summary of the results of cytogenetics assays that had been caried out on 5 naphthenic and 2 paraffinic base oils. A full description for one of the studies is given above. The results for all seven samples are summarized briefly here.

Result : The results tabulated in the publication are as follows:

Sample	Dose (mg/kg)	No. animals	No. cells	Aberrant cells (%)
Paraffinic oils				
64 SUS	Corn oil	8	400	4.3
	500	10	500	3.8
	1000	9	450	2
	2000	10	500	2.8
133 SUS	Corn oil	10	500	3
	500	8	400	1.3
	1000	10	500	2
	2000	10	500	1
331 SUS	Corn oil	10	500	4
	500	9	450	3.8
	1000	8	450	5.6
	2000	10	500	7*
485 SUS	Corn oil	7	350	4
	500	9	450	4.9
	1000	8	400	4.3
	2000	7	350	5.7
990 SUS	Corn oil	8	400	1
	500	6	300	1.3
	1000	9	450	1.6
	2000	8	400	2.5
Naphthenic oils				
80 SUS	Saline	19	950	0.4
	500	17	850	0.4
	1670	19	950	0.6
	5000	20	1000	0.4
2000 SUS	Saline	19	950	0.7
	500	18	874	0.7
	1670	18	900	1.6
	5000	15	750	0.4
TEM	0.4-1.0			24.2-41.8*

* denotes significant by Wilcoxon rank test

Source : The data summarized by Conaway et al originated from an API program. The reports from which the data were summarized are:

	API Report No
Paraffinic oils	.
64 SUS	28-31864
133 SUS	28-31868
331 SUS	28-31865
485 SUS	28-31866
990 SUS	28-31867
Naphthenic oils	
80 SUS	29-32359
2000 SUS	29-32360

Test substance : Two naphthenic and 5 paraffinic base stocks were tested. The characteristics of the samples tested are as follows:

Sample	Initial boiling point (° F)	Aromatics (%)	PNAs (%)
Paraffinic oils			
SUS at 100 °F			
64	536	10.2	0.4
133	639	13.8	0.7
331	636	28.1	3.0
485	572	27.8	4.1
990	515	31.9	4.8
Naphthenic oils			
SUS at 100 °F			
80	470	23.8	0.8
2000	611	37.7	4.5

Reliability : (1) valid without restriction

(78)

5.7 CARCINOGENICITY

Species : Mouse
Sex : Male/female
Route of admin. : Dermal
Exposure period : Up to 84 weeks
Frequency of treatm. : Once or twice weekly
Doses : Various
Control group : Yes, concurrent no treatment
Test substance : Distillate base oils

Remark : Numerous skin carcinogenicity studies have been carried out on lubricating base oils derived from distillates. Data from these studies have been summarized and reviewed elsewhere.
No single study is summarized here but the general conclusions that may be drawn from the numerous studies are:

Highly refined base oils are not skin carcinogens.

Poorly refined or unrefined base oils are skin carcinogens.

A good correlation exists between skin carcinogenic potential and

level of DMSO extractables and polycyclic aromatic compounds present in the base oil.

The degree of carcinogenicity is dependent on the level of polycyclic aromatic compounds present in the base oil.

When applied repeatedly to the skin, carcinogenic base oils are associated only with skin tumors and not with an increase in systemic tumors.

There is a good correlation between skin carcinogenicity and Mutagenicity Index as determined in a modified Ames assay.
(23) (26) (80) (81) (101) (111)

Species	:	Mouse
Sex	:	Female
Strain	:	CF No. 1
Route of admin.	:	Dermal
Exposure period	:	18 months
Frequency of treatm.	:	Three times weekly
Doses	:	0.1ml/application
Result	:	Negative
Control group	:	Yes
Year	:	1991
GLP	:	No data
Test substance	:	Residual base oils (See below)

Method : 0.01 ml of undiluted test material was spread three times weekly over the shorn dorsal skin of a group of 50 female CF No.1 mice. A further two groups of 5 female mice underwent similar treatment and were killed after 22 or 52 weeks.

The appearance and development (or regression) of superficial tissue masses was recorded weekly throughout the study, to enable calculation of the latency period of those subsequently diagnosed as being tumors.

A positive control group of 50 female mice was treated with an oil (N1) that had been shown in previous studies to be a skin carcinogen. The mice in the positive control group received the oil once a week for 22 weeks and then once every 14 days for a total of 78 weeks.
A group of 50 untreated female mice served as negative controls.

Result : Minimal evidence of skin irritation was visible following treatment with the test materials.
No treatment-related effects were observed on clinical condition, body weight gain or mortality (NB survival rates for treated animals are not included in the report).
Changes recorded at post mortem were considered normal.
Histopathological examination of the skin of the treated mice provided no evidence of skin irritation and no tumors of epidermal origin were observed.

No cutaneous tumors were recorded in the group of untreated control mice (52% of animals survived to termination after 2 years)

The positive control group had skin reactions at the treatment site which included redness, scabbing, cracking and flaking; histopathological examination confirmed the presence of chronic inflammation (acanthosis, hyperkeratosis, ulcers, parakeratosis and scabs). In addition, skin

reactions, principally at the margins of the treatment site were frequently recorded and were particularly seen during the first 22 weeks of treatment. These reactions typically included abrasions and ulceration. The severity of the lesions was such that many animals were killed on humane grounds; only 24% of animals survived to 78 weeks.
Histopathological examination of the skin revealed that over 78 weeks, 23 mice in the positive control group had 56 tumors of epidermal origin, of which 39 were benign (papillomas and keratoacanthomas) and 17 were malignant (squamous cell carcinomas and one single malignant basal cell tumor). The mean latency period was 37 weeks.

Test substance : The test substance was described as:
"A non-solvent refined, deasphalted, dewaxed residual paraffinic lubricant base oil"

Characteristic	Value
Kinematic viscosity	
at 40 °C	1024 cSt
at 60 °C	266.6 cSt
at 100 °C	42.52 cSt
Density at 15 °C	0.9280 kg/l
Pour point	+3 °C
Flash point (COC)	315 °C
Refractive index	1.5142
Color (D1500)	8.0
Molecular weight (D2502)	660
Sulfur	1.7% wt
Aniline point	105.0 °C
Volatiles 3 hrs at 13 °C	0.10%
Neutralization value	0.02 mg KOH/g
Viscosity gravity constant (D2140)	0.846
Refractivity intercept	1.0598
Molecular type (D2007)	
Saturates	46.3% wt
Aromatics	45.6% wt
Polars	8.0% wt
Carbon type (D2140)	
CA	15%
CN	19%
CP	66%

Total and individual PCA concentrations on completion of study

Individual PCA	mg/kg
Fluoranthene	0.2
Pyrene	0.9
Benz(a)anthracene	0.3
Chrysene/triphenylene	2.5
Benzofluoroanthenes	1.0
Benzo(e)pyrene	1.6
Benzo(a)pyrene	0.1
Perylene	0.1
Dibenz(a,j)anthracene	<0.1
Dibenz(a,h)anthracene	<0.1
Indeno(1,2,3-cd)pyrene	<0.1
Benzo(ghi)perylene	<0.1
Total PCA content (BP3 method)	7.0% wt

Reliability : (4) not assignable

This report is a summary report and as a consequence does not provide full experimental details, but does provide sufficient information for a conclusion to be made on the skin carcinogenic potential of a non-solvent refined residual paraffinic base oil.

(103)

Species	:	Mouse
Sex	:	Male
Strain	:	C3H
Route of admin.	:	Dermal
Frequency of treatm.	:	3 times weekly
Doses	:	25 µl per application
Result	:	Negative
Control group	:	Yes
GLP	:	No data
Test substance	:	Canthus 210 a Deasphalted, dewaxed, residual oil
Method	:	The summary states that the design of the study was similar to other conventional skin painting studies in mice. The test material was applied undiluted in 25 µl aliquots to the clipped dorsal back regions of 50 male C3H/HeJ mice, three times weekly. At each treatment period, the dorsal skin was examined for the presence of papillomas/carcinomas, mined daily for any clinical signs of ill health. Treatment continued for 24 months. A complete necropsy was conducted at the time of sacrifice. In this study, Primol 185, a medicinal grade white mineral oil was applied undiluted and served as the negative control. Heavy Clarified Oil (HCO) was applied as a 10% solution in Primol 185, and served as the positive control.
Result	:	None of the animals treated with the test material or the negative control material developed skin tumors, or any other tumors considered treatment-related, over the course of the study. The positive control material, 10% HCO, responded as anticipated, producing squamous cell carcinomas in 47 of 50 treated animals.
Reliability	:	(4) not assignable The information given is based on a summary of the study and hence it is not possible to assign reliability to the study. Nevertheless, the data provide useful information on the carcinogenic potential of residual base oils.

(87)

Species	:	Rat
Sex	:	Male/female
Strain	:	Fischer 344
Route of admin.	:	Oral feed
Exposure period	:	2 years
Frequency of treatm.	:	Daily in the diet
Doses	:	60, 120, 240 and 1200 mg/kg/day
Result	:	Negative
Control group	:	Yes
Method	:	OECD Guide-line 453 "Combined Chronic Toxicity/Carcinogenicity Studies"
Year	:	2001
GLP	:	Yes
Test substance	:	White oil
Remark	:	This study is a study that was conducted according to OECD guidelines. It is not described in full in this summary since it is not one of the SIDS base

set requirements.

Result : Survival was unaffected by exposure to the test material. There were no treatment related clinical signs, or any effects on body weight, food consumption, food conversion efficiency or ophthalmology. Furthermore, there was no treatment related effects on the hematological, serum chemistry or urinalysis parameters that were measured. At gross necropsy, there were no treatment-related gross observations and there were no treatment-related neoplastic changes.

Test substance : The test material is a 70 cSt white oil with an average molecular weight of 485.

Reliability : (1) valid without restriction

(96)

Species : Rat
Sex : Male/female
Strain : Fischer 344
Route of admin. : Oral feed
Exposure period : 104 weeks
Frequency of treatm. : Continuous in the feed
Doses : 2.5 and 5% in the diet
Result : Negative
Control group : Yes
Year : 1997

Result : There were slight increases in body weights in both sexes of the 5% group (5% for males and 2.7% for females) at week 104. Food consumption was also increased in the 5% groups (11% for males and 8% for females total increase at week 104). However, no significant treatment-related differences between the control and treated groups were observed for clinical signs, mortality or hematological findings.
In the 5% group, absolute liver and kidney weights were increased in males and absolute and relative submaxillary gland weight were reduced in females. Absolute and relative weights of heart and spleen were unaffected by treatment.
The percentage increases/decreases in the 5% group were:

Organ	Absolute	Relative
Female		
Submaxillary gland	3% decrease	1.7% decrease
Male		
Liver	8.4% increase	not different
Kidney (R)	14.9% increase	not different
Kidney (L)	9.9% increase	not different

In the 5% male group, the increased absolute organ weights were attributed to the slight increases in body weights.

A variety of tumors developed in all groups, including the control group. However, all the neoplastic lesions were histologically similar to those known to occur spontaneously in F344 rats, and no statistically significant increase in the incidence of any tumor type was found for either sex in the treated groups.

Granulomatous inflammation in the mesenteric lymph nodes, considered to be a reaction to paraffin absorption, was observed with similar incidence and severity in both sexes of the 2.5 and 5% groups.

The authors concluded that under the present experimental conditions, the high dose, about 2000-200,000 times higher than the current temporary acceptable daily intake, did not have any carcinogenic potential in F344 rats. Furthermore, the granulomatous inflammation observed in the mesenteric lymph nodes was not associated with any development of neoplastic lesions.

Test substance : The test material was composed of equal quantities of eight different commercially available liquid paraffins (highly refined white oils) obtained from eight member companies of the Japan Liquid Paraffin Industry.
Each of the eight liquid paraffins complied with the requirements of the Japanese food additive and Japanese Pharmacopoeia standards. 5 of the component material had been derived from petroleum by acid treatment and the other eight had been derived by hydrotreatment.
The physical properties of a sample of the composite test material were determined by CONCAWE and were as follows:

Viscosity at 40°C	0.871
Viscosity at 100 °C	8.68
Ratio of naphthenic/paraffinic hydrocarbon	35/65
Average molecular weight	475
Carbon No. at 5% boiling point	25

Reliability : (2) valid with restrictions
Although the experimental details are not provided here, the information is nevertheless useful in establishing the lack of carcinogenicity by the oral route.

(119)

5.8.1 TOXICITY TO FERTILITY

Type : One generation study
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Gavage
Frequency of treatm. : Daily
Doses : 1.15 mg/kg
Control group : No
Method : OECD Guideline 421, Reproductive/Developmental Toxicity screening test
Year : 1995
GLP : Yes
Test substance : Chevron 100 neutral (refined) CAS 64742-54-7

Method : The method used was as described in OECD guideline 421.

The base oil was administered by gavage at a dose of 1.15 mg/kg (bw) to a group of 12 male and 12 female Sprague Dawley rats. Rats designated F0 animals were dosed for a minimum of 14 days prior to mating. Dosing was continued after mating until a total dosing period of 30 days had elapsed for males and until day 4 of lactation for females (39 days). The animals were observed twice daily for appearance, behavior, moirbundity and mortality. Males and females were also observed during dosing and for one hour thereafter.
Male F0 body weights were recorded weekly. Female F0 body weights were also recorded weekly until evidence of mating was observed and then on gestation days 0, 7, 14 and 20 and on lactation days 1 and 4. Food consumption was also recorded for F0 both sexes.

Animals were paired on a 1:1 basis. Positive evidence of mating was confirmed either by the presence of sperm in a vaginal smear or a vaginal plug. The day when evidence of mating was identified was termed Day 0 of gestation.

The following Fertility indices were calculated:
Female mating index
Male mating index
Female fertility index
Male fertility index

All females were allowed to deliver their young naturally and rear them to post-natal day 4. Females were observed twice daily during the period of expected parturition for initiation and completion of parturition and for signs of dystocia. After parturition, litters were sexed and examined for evidence of gross malformations, numbers of stillborn and live pups.
Litters were examined daily and each pup received a detailed physical examination on days 1 and 4 of lactation. Any abnormalities were recorded.
The live litter size and viability index were calculated.
All surviving pups were necropsied on post-natal day 4.
A complete gross examination was made on all animals at necropsy.
Selected organs of parental animals were weighed and a wide range of tissues was fixed for subsequent histopathological examination.

Result : Only the results for the base oil control group are reported below.

There were no clinical findings and growth rates and food consumption values were normal.
Fertility indices and mating indices for males and females were both 100%.
At necropsy, there were no consistent findings and the animals were considered to be normal.
Organ weights and histopathology was considered normal.

Reliability : (2) valid with restrictions
The study was on an oil additive in base oil at two concentrations. The base oil alone was used as the control. Therefore, no control was available with which to compare the study control group. However, since all the recorded values were within normal limits, it could be concluded that the base oil was without effect.

(128)

Type : One generation study
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Gavage
Exposure period : 13 weeks prior to mating
Frequency of treatm. : 5 times weekly
Premating exposure period
Male : 13 weeks
Female : 13 weks
Duration of test : One generation after 13 weeks dosing
No. of generation studies : 1
Doses : 5 ml/kg
Control group : No
Year : 1987
GLP : No data

Test substance : White oil CAS 8012-95-1

Method : 72 female and 36 male Sprague-Dawley rats were given white oil at a dose of 5 ml/kg, 5 days a week for 13 weeks. After this time each of the males was housed with 2 females for 10 consecutive nights, or until mating was confirmed by the appearance of a copulatory plug or by the presence of sperm in a vaginal rinse.
The mated females were maintained without further dosing through gestation and lactation to post-partum day 21.
Detailed maternal physical examinations and body weight measurements were made on days 0, 7, 14 and 21 of gestation and on days 0, 4, 14 and 21 of lactation.
All dams and surviving litters were sacrificed and grossly examined on day 21 of lactation. Each of the offspring was examined for external malformations. All pups were then sacrificed, necropsied and subjected to visceral organ and brain examination. Pups which died spontaneously were also necropsied unless this was precluded by cannibalism or aut

Remark : White oil was used as solvent control in a study to determine the effects of two EDS coal liquids in a 13 week subchronic a single generation reproduction study. There were three dose groups and a control group for each test material in this study. The information in this robust summary relates only to the white oil control groups (one for each of the test materials) and NOT to the groups exposed to EDS coal liquids.

The CAS# for the material that was used in this study is not included in the Lubricating Base Stocks category. However, because white oils are so highly purified, toxicologically and compositionally they are all very similar. Therefore, the Testing Group thinks the results on CAS # 8012-95-1 are applicable to the highly refined base oils that are included in this category.

Result : The data for the two control groups are summarized below.

Parameter	Control 1	Control 2
Impregnation frequency	80.8%	80.9
Gestation	22.6 days	22.6
Pups delivered	11.7	11.1
Live births	11.2	10.7
Survival at day 4	10.5	9.6
Survival at day 14	10.2	9.3
Survival at day 21	10.1	9.3
Offspring body weights		
Day 0 lactation	6.7	6.9
Day 4 lactation	9.3	9.9
Day 14 lactation	26.9	27.1
Day 21 lactation	43.2	46.7

No unusual behavior was reported during the gestation period for either of the control groups. The general condition of offspring and dams was good through weaning.
Gross observations of pups and dams were generally unremarkable.
In one base oil group, 3 malformed pups were found in 2 litters. Two of the malformed pups had syndactyly and renal agenesis and one of these also exhibited agnathia. The third pup had a small eye.

In the other control group, four malformed pups were found in 4 litters.

Two of the pups had tail abnormalities, one had a depression in the sternum and the fourth had a short snout.

The authors comment that a similar spectrum of malformations in Sprague-Dawley rats from the same supplier has been reported elsewhere. The authors also comment that this spectrum of malformations can occur spontaneously in the Sprague-Dawley rat and are not regarded as treatment-related.

Test substance : The test substance is not listed in the US HPV program.
Nevertheless, it is a white oil and the results are directly applicable to other highly refined white oils.

Reliability : (2) valid with restrictions
Not all the raw data are presented in this publication. However, the data are useful in determining that white oils do not cause effects on reproduction after prior exposure
for 13 weeks.

(105)

Type : Reproduction/developmental study
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : 14 days premating to day 20 of gestation
Frequency of treatm. : Daily
Premating exposure period
Male : 14 days
Female : 14 days
Doses : 1 ml/kg
Control group : Yes
Method : OECD Guide-line 421
Year : 1997
GLP : No data
Test substance : Mineral oil USP

Method : The study was performed in accordance with OECD guideline 421 with the addition that males were treated for 8 weeks to improve observation of effects on the reproductive system. Also females were weighed 7 times during gestation rather than 4, and at necropsy, 7 organs in addition to the reproductive organs were weighed.

Ten approximately eight week old male Sprague Dawley rats (275-285g) and 10 females of the same age (183-187g) were treated dermally with kerosene at concentrations of 20, 40 or 60% (v/v) in mineral oil in a dosing volume of 1 ml/kg. These doses were selected on the basis of the results of a preliminary 2-week range finding study.
In addition There were two control groups: the vehicle control was given mineral oil only at a rate of 1 ml/kg/day and in the sham-treated group the animals had been fitted with collars and were stroked with the tip of a syringe, but no material was applied.

Test material or mineral oil was applied daily to the shorn skin of the animals 7 days/week from 14 days premating, during 14 days mating and through 20 days of gestation. Collars were fitted to the animals during the dosing period to prevent ingestion of applied materials. After the final dose, the collars were removed and residual test material was wiped from the skin. Males continued treatment through gestation until final female

sacrifice on days 4-6 of lactation.

During the mating period the test material remained on the animal's backs for 6 hours. Prior to pairing, the test material was removed by wiping. Rats were mated overnight on a 1:1 ratio and were separated the following morning. Collars were then applied prior to the next dose being applied. Females were monitored for evidence that mating had taken place. Pregnancy was determined by the presence of a vaginal plug or sperm in a vaginal lavage sample. If observed, the female was considered to be at day 0 of gestation. Any female that did not show evidence of mating was placed with the same male the following evening. Any female that did not show evidence of mating at the end of a 2 week mating period was presumed pregnant (gestation day 0 = last day of cohabitation).

Animals were checked twice daily for morbidity and mortality during weekdays but only once daily at weekends. Animals were also observed immediately prior to dosing and after the last animal had been dosed for appearance, behavior and motor activity, respiratory function, central nervous system function, excretory function and biological discharges. Effects of test material on the skin were assessed and scored weekly, using Draize scales for erythema and edema and for chronic deterioration. Males were weighed on the first day of dosing, then weekly and on the day of sacrifice. Females were also weighed on the first day of dosing, then weekly until mating was confirmed and thereafter on gestation days 0, 3, 6, 10, 13, 16 and 20 and on post partum days 0 and 4. Food consumption was also monitored on a similar schedule except through the mating period.

Each presumed-pregnant female was observed daily from gestation day 20 for parturition; evidence of dystocia was noted. The day of delivery was designated postpartum day 0. Maternal behavior and appearance were monitored daily until sacrifice.
Each litter was examined as soon as possible after birth to establish the number and sex of pups, stillbirths, live births and the presence of gross abnormalities. Pups were examined daily for presence of milk in their stomachs. Any pup found dead was examined externally and unusual findings were recorded. The body weight of each viable offspring was individually measured and recorded on post partum days 1 and 4.

Adult females that did not deliver were sacrificed on day 25 of gestation. Dams that delivered and maintained their litters until post partum day 4 were sacrificed with their offspring on post partum days 4-6.
All males were sacrificed after the females had been killed.
All animals were examined macroscopically for structural anomolies and pathological changes, with emphasis on the reproductive organs. The numbers of implantation sites and corpora lutea of each adult female was recorded. No tissues from offspring were retained.

The liver, kidneys, adrenals, thymus, spleen, brain and heart of all parental animals were weighed. In addition the testes and epidiymides of parental males were weighed.
Skin from treated sites, ovaries and testes and epididymides were prepared for histological examination. Pathological evaluation was performed on reproductive organs from all males and pregnant females in both control groups and the high dose group and on treated skin from all groups.

Statistical evaluation
Quantitative data (body weight and food consumption) were analyzed by parametric methods: analysis of variance (ANOVA) and associated F-test, followed by Dunnet's test for multiple comparisons, provided there was statistical significance in the ANOVA. Maternal reproductive data were evaluated by ANOVA followed by group comparisons using Fisher's exact test. Differences between control and treatment groups were considered statistically significant only if the probability of the differences being due to chance was less than 5% (P< 0.05).

Remark : This is a study of the effects of kerosine and two control groups were used:
1 Sham-treated control
2 MIneral oil applied at 1 ml/kg/day

Only the results relating to mineral oil are presented in this robust summary.

Result : Only the results of the untreated control group and the group given mineral oils are summarized below.

No animals died or were prematurely sacrificed and no clinical signs of toxicity were observed.
Skin irritation among males varied from slight to moderate with increasing dose and was most severe in the high dose group. Mild to moderate skin irritation was observed in females at the highest concentration.
At terminal sacrifice, no findings were reported except for those on the skin. Microscopic changes were found in the skin of vehicle control and all kerosine-treated groups in the males. In females changes were only observed in the high dose group animals. The skin findings (macroscopic and microscopic) are shown in the following table.

Parameter	Control	Mineral oil
Males		
No animals	10	10
Max. skin irritation score,		
Week of max severity		
	-	2
Mean (SD)	0	1.3 (1.2)
Min/max score	0	0/3
Gross necropsy observations		
Crust/scab	1	0
Scaly/dry/flaky	0	0
Histopathological observations		
Acanthosis/hyperkeratosis	2	5
Hyperplasia, sebaceous glands	3	5
Inflammation, dermal	2	1
Necrosis, epidermal, focal	1	0
Females		
No animals	10	6
Max. skin irritation score, sum of means		
Week of max severity		
	6	7
Mean (SD)	0.2(0.6)	0.7(1.0)
Min/max score	0/2	0/2

Gross necropsy observations		
Crust/scab	0	1
Scaly/dry/flaky	0	0
Histopathological observations		
Acanthosis/hyperkeratosis	3	2
Hyperplasia, sebaceous glands	1	0
Inflammation, dermal	0	1
Necrosis, epidermal, focal	0	0

Body weights were unaffected by treatment.

Reproductive/fertility data are shown in the following table

	Controls	
Parameter	**Sham-treated**	**Mineral Oil**
No animals	10	10
Fertility index	100%	90%
Litter with liveborn pups	10	9
Corpora lutea		
Number	169	151
Mean (SD)	16.9 (1.9)	16.8 (2.4)
Implantation sites		
Number	163	149
Mean (SD)	16.3 (1.9)	16.6 (2.4)
Pups delivered		
Total	152	131
Mean (SD)	15.2 (2.0)	14.6 (2.7)
Liveborn	152	130
Livebirth index	100%	99%
Pups dying		
day 0	3	0
days 1-4	2	4
Pups surviving		
4 days	147	126
Viability index	97	97
Pup weight/litter (g)		
day 1 mean	6.9	6.8
day 4 mean	9.9	9.6

No test-material-related microscopic changes were observed in the testes or epididymides of adult male rats or in the ovaries of adult female rats.

Reliability : (1) valid without restriction

(113)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Gavage
Exposure period : Days 6 through 19 of gestation
Frequency of treatm. : Daily
Year : 1987
GLP : No data
Test substance : White oil CAS 8012-95-1

Method : Two groups of animals (50 and 25)were administered white oil by gavage at a dose of 5 ml/kg, every day during gestation days 6 to 19 inclusive. Food and water were available continuously. Animals were examined for viability and clinical effects twice daily. Body weights were recorded on days 0, 6, 10 and 20 of gestation.
On day 20 of gestation, all animals were euthanized with methoxyfluorane and examined for gross changes. Each gravid uterus was removed and weighed. The number, location and viability of each fetus and the number of implant sites were recorded. Fetuses were removed, weighed and the crown-rump lengths measured. All live and dead fetuses that had not been resorbed were examined for external malformations. Approximately half of the fetuses from each litter were decapitated and the heads preserved for subsequent examination for abnormalities. The viscera were also examined for malformations under low power magnification. The remaining fetuses were stained with Alizarin red and subsequently examined for skeletal abnormalities.
No organs, other than the uteri were weighed and no organs were examined histologically in this study.

Remark : White oil was used as the solvent control in two separate studies, one for each of two test materials. This summary only reports on the outcome of the animals in ps.

The CAS# for the material that was used in this study is not included in the Lubricating Base Stocks category. However, because white oils are so highly purified, toxicologically and compositionally they are all very similar. Therefore, the Testing Group thinks the results on CAS # 8012-95-1 are applicable to the highly refined base oils that are included in this category.

Result : One animal died in the control group containing 50 animals and this was attributable to misdosing.
Increases in body weight during the study were considered summarized in the table below.

Day of gestation	**Group 1 (25 rats)**	**Group 2 (50 rats)**
Body weights (g)		
0	207.2	225.4
6	227.5	248
10	235.9	259.3
15	260	284.3
20	329.1	351.9
Uterine wt	67.2	70.7
Number of litters	25	49
Implants/litter	11.3	12.0
Resorptions/litter	0.06	0.47
Males		
No./litter	5.12	5.96
Crown-rump length (mm)	3.66	3.6
Wt. of fetuses	4.26	4.23
Females		
No./litter	5.6	5.61
Crown-rump length (mm)	3.61	3.52
Wt. of fetuses	4.02	4.07

In the control group containing 50 animals, 3 malformed fetuses were found in 3 litters; one had an extra lumbar vertebra, one had a discrete area of ossification in the area of the junction of the frontal and nasal

bones, one had moderately dilated lateral ventricles of the brain.

3 malformed fetuses were also found in 3 litters of the other control group. These were, a vertebral arterial canal of a cervical process fully ossified in 2 fetuses and angulated ribs in a third fetus.

The authors considered these malformations to be minor and that the findings were within the normal ranges for the strain of rat.

Reliability : (2) valid with restrictions
Although there were no untreated animals for comparison, the results were nevertheless, considered to be within normal limits. Consequently, the study is useful in providing evidence of the lack of developmental effects for white oil.

(104)

5.11 ADDITIONAL REMARKS

Type : Correlation of toxicity with chemical components of refinery streams

Remark : Heavy vacuum gas oil is used as a starting material for base oil production. As such, it can be considered a "worst case" example of the unrefined/mildly refined base oil subcategory. Studies on this material are summarized below.

Type : 90-day study on Heavy vacuum gas oil

Method : Undiluted heavy vacuum gas oil was applied at doses of 0, 30, 125, 500 and 2000 mg/kg/day to the shorn skin of groups of ten male and ten female Sprague Dawley rats. The males weighed between 220 and 230 g and the females weighed between 160 and 170 g at the start of the study.
The material was applied 5 days each week for 13 weeks. Collars were fitted to the animals to prevent oral ingestion.
Body weights were recorded weekly throughout the study and clinical observations were made daily. Skin irritation was assessed weekly. At 5 and 13 weeks, blood samples were taken for measurement of the following hematological and clinical chemical parameters:

<u>Hematology</u>

Red blood cell count	Hemoglobin
Hematocrit	White blood cell count
Differential WBC count	MCV, MCH & MCHC caclulated

<u>Clinical chemistry</u>

Glucose	Urea nitrogen
Uric acid	Total protein
Albumin	Globulin (calculated)
Albumin/Globulin ratio	Calcium
Alkaline phosphatase	Alanine aminotransferase
Aspartate aminotransferase	Lactate dehydrogenase
Sorbitol dehydrogenase	Creatinine
Cholesterol	Triglycerides
Total Bilirubin	Calcium
Phosphorus	Sodium
Potassium	Chloride

At the end of the study (13 weeks) all surviving animals were sacrificed and a gross necropsy examination was performed. The following organs were weighed:

Adrenals	Kidneys	Spleen
Brain	Liver	Testes
Epididymes	Ovaries	Thymus
Heart	Prostate	Uterus

The following tissues in the high dose group animals were examined microscopically:

Adrenals (both)	Ovaries (both)
Bone & marrow (sternum)	Pancreas (head)
Brain (3 sections)	Salivary gland (submaxillary)
Eye & optic nerve	Skin (treated, 2 sections)
Heart	
Colon	Duodenum
Stomach	Kidneys (both)
Testes (both)	Liver (2 lobes)
Thymus (both lobes)	Lung (left lobe)
Thyroid (both lobes)	Muscle (skeletal, thigh)
Urinary bladder	Peripheral nerve (sciatic)
Gross lesions	

Histopathological examination was only undertaken on thymus, spleen and sternum for the 500 mg/kg/day animals and thymus only for the 125 mg/kg/day animals.

Result : Two males and one female in the high dose group died during the study. The male deaths were considered to be compound related but the female death was considered incidental. Growth rates of males and females in the highest dose group were reduced compared to controls. At 13 weeks the males weighed 20% less and the females 15% less than controls. At 2000 mg/kg/day males and females had reduced erythrocytes and reduced platelets at 5 and 13 weeks. Similar effects were also found in the 500 mg/kg/day females.

Clinical chemical changes in males and females at 2000 mg/kg/day consisted of:

- twofold increase in sorbitol dehydrogenase
- twofold increase in cholesterol
- 50% reduction in uric acid

In addition in females at 500 mg/kg/day, glucose was reduced and in the 500 mg/kg males cholesterol was increased.

At gross necropsy, relative thymus weights were reduced in the 500 (by 25%) and 2000 mg/kg/day (by 50%) animals of both sexes. Relative liver weights were also increased at 500 and 2000 mg/kg/day for both sexes.

Histological examination revealed decreased erythropoeisis and fibrosis of the bone marrow in the 2000 mg/kg/day males.
There was a reduction in thymic lymphocytes in the 2000 mg/kg/day groups (marked for males and moderate for females) and a slight reduction in the 500 mg/kg/day groups for both sexes.

No effects were found on either sperm morphology or in the results of the urinalysis.

The NOEL for both males and females was found to be 125 mg/kg/day.

Test substance : The sample of Heavy vacuum gas oil was produced by the vacuum distillation of crude oil.
It was a dark amber liquid with a boiling range of approximately 657 to 1038 °F.
The sample originated from the Beaumont crude unit B (CRU #85244) and contained:
54% paraffins
35% polycyclic aromatic hydrocarbons
2% nitrogen-containing polycyclic aromatic hydrocarbons
9% residuals.

Reliability : (2) valid with restrictions
The report evaluated was incomplete but nevertheless was sufficient to identify the relevant effects of exposure to the test material.
(106)

Type : Developmental toxicity screen on Heavy vacuum gas oil

Method : Groups of 10 presumed-pregnant rats (approximately 9-10 weeks old) were distributed into the following groups:

Group	Dose level (mg/kg/day)	Gestation days of administration
1	0 (remote control)	0-19
2	0 (proximate control)	0-19
3	30	0-19
4	125	0-19
5	500	0-19
6	1000	0-19
7*	500 (bioavailability)	10-12

* Group size was 5 at start but increased to 8 after study initiation.

The test material was applied daily to the shorn dorsal skin at the dose levels shown above and for the duration indicated. The rats were fitted with collars to prevent oral ingestion of the applied material. Since it was believed that inhalation of test material could be a confounding factor a second group of controls (remote controls) were housed in an area in which they could not inhale gasoil that had been applied to other animals.

Observations were made daily for clinical signs and body weights and food consumption were recorded regularly throughout the study.

Each female was sacrificed on day 20 of presumed gestation and the thoracic and abdominal cavities were examined grossly. The thymus and liver were removed from each animal and weighed and then preserved in formalin but not examined further.
The uterus and ovaries were removed and examined grossly. The number of corpora lutea per ovary for each rat was recorded. The ovaries of non-pregnant females were examined and then discarded. Uterus weights were also determined.
The uterine contents of each pregnant rat were exposed and a record made of the number and location of all implantations.
At necropsy, blood samples were taken from all the animals and a range of clinical chemical measurements were made of the following:

Alanine aminotransferase (ALT)	Glucose
Albumin	Iron

Albumin/globulin ratio	Phosphorus, inorganic
Alkaline phosphatase (ALP)	Potassium
Bilirubin, total	Sodium
Calcium	Sorbitol dehydrogenase (SDH).
Chloride	Total protein
Cholesterol	Triglycerides
Creatinine	Urea nitrogen
Globulin	Uric acid.

Fetuses were examined and half were preserved in Bouin's solution for examination of soft tissue abnormalities, the remainder were being differentially stained for subsequent skeletal examination.

Statistical analysis
Maternal biophase and cesarean section data and fetal data were evaluated statistically by analysis of variance followed by group comparisons using Fisher's Exact or Dunnet's Test.
Fetal skeletal and visceral data were evaluated statistically by ANOVA followed by group comparisons using Fisher's Exact test.
Thymus and liver weights were evaluated statistically using Student-Newman-Keul's test.
Statistical analyses of clinical chemistry data were performed separately on individual serum components using SAS procedures. First the F-test was employed to do an analysis of variance on the serum data obtained from control and exposed groups. Next, the Student-Newman-Keul's multiple comparison test was employed to identify the specific group subsets within the serum data sets identified as having nonrandom variance.
In general, for all statistical tests, differences between control and treated groups were considered statistically significant if the probability of the difference being due to chance was less than 5% ($P<0.05$).

Result : Parental animals.
There were no clinical signs attributable to exposure to HVGO other than in the highest dose group in which 2 rats had a red vaginal discharge, one animal was pale in color and six had decreased stool. The latter observation was probably associated with smaller food consumption in this group. Although food consumption was generally also less than controls in the 500 mg/kg/day group there was no associated body weight decrease.
At doses in excess of 125 mg/kg/day there was a decrease in mean body weights which reflected the decreased litter sizes for this group. The only dose-related finding at gross necropsy was a pale appearance of lungs in a few animals. 4 animals were affected at the highest dose and only one in the 500 mg/kg/day group.
Mean thymus weights of animals in the highest dose group were approximately half those of the control groups.
Although absolute liver weights were unaffected by exposure to HVGO, mean relative liver weights were increased (approximately 15%) in groups exposed to doses greater than 125 mg/kg/day.

Observations of Dams at Caesarean section.
Parameters with treatment-related effects are shown below.

Dose group (mg/kg/day)

0(R)	0(P)	30	125	500	1000
Pregnant females					
9	10	10	8	10	9
Dams with viable fetuses					

	9	10	10	8	10	6
Dams with all resorptions						
	0	0	0	0	0	3
Mean litter size of viable fetuses						
	13.9	14	13.8	14.4	10	5.8
Resorptions						
Mean	1.1	0.6	1.1	1.1	5.6	9.9
% Dams with resorptions						
	56	50	70	63	100	100

Parameters unaffected were:
- No. premature births
- Female mortality
- No. corporea lutea
- No. implantation sites
- Pre-implantation losses
- Viable male fetuses
- Viable female fetuses
- No. dead fetuses

Fetal evaluations

fetal body weights were significantly reduced in fetuses exposed in utero to HVGO at doses in excess of 125 mg/kg/day.
Although there were differences between control and treated crown-rump lengths they were not statistically significant.
At the time of external examination, malformations were observed in one fetus in the 1000 mg/kg/day group. The fetus was edematous and pale in color. Both hindpaws were ced in size with a spect of each of the digits.
Malformations of the vertebral column were restricted to the 500 mg/kg/day group.
Although a variety of skeletal malformations were observed in treated and control groups the degree of aberrant development in control fetuses was not as severe as in the HVGO-exposed groups.
Visceral malformations were restricted to two fetuses in the 500 mg/kg/day group. One fetus had microphthalmia and the other fetus had a diaphragmatic hernia which displaced the heart from the left to right hand side.

The authors concluded that the maternal NOAEL was 125 mg/kg/day and that the fetal NOAEL was also 125 mg/kg/day

Test substance : The sample of Heavy vacuum gas oil (CAS 64741-57-7) was produced by the vacuum distillation of crude oil.
It was a dark amber liquid with a boiling range of approximately 657 to 1038 °F and density 0.93 g/ml.
The sample (CRU #85244) originated from the Beaumont crude unit B and contained:

54%	paraffins
35%	polycyclic aromatic hydrocarbons
2%	nitrogen-containing polycyclic aromatic hydrocarbons
9%	residuals

Reliability : (2) valid with restrictions
The report evaluated was incomplete but nevertheless was sufficient to identify the relevant effects of exposure to the test material.

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ATTACHMENT 1: Physico-chemical properties for selected lubricating oil basestocks

Base oil description	Kinematic viscosity * at 40°C (mm^2/s) ASTM D445	Kinematic viscosity * at 100°C (mm^2/s) ASTM D445	Flash Point (°C) ASTM D93	Pour Point (°C) ASTM D97	Density (kg/l) ISO 12185	Average Molecular Weight ASTM D2502
Distillate oils						
Solvent-dewaxed, light paraffinic (64742-56-9)	8.4	2.4	157	-18	0.85	280
Solvent-dewaxed, heavy paraffinic (64742-65-0)	25.1	4.8	204	-12	0.86	390
Hydrotreated, light paraffinic (64742-55-8)	17.0	3.7	190	-18	0.86	360
Hydrotreated, heavy paraffinic (64742-54-7)	73.9	9.1	232	-9	0.88	500
Hydrotreated, light naphthenic (64742-53-6)	8.5	2.2	145	-60	0.87	290
Hydrotreated, heavy naphthenic (64742-52-5)	145	10.5	220	-24	0.91	440
White mineral oil (8042-47-5)	27.3	5.0	217	-15	0.86	400
Residual oils						
Solvent-dewaxed (64742-62-7)	1300	50	285	-6	0.95	700

* Kinematic viscosity is often expressed in Centistokes (cSt). It should be noted that 1 cSt = $1mm^2$/second.

ATTACHMENT 2: Summary of repeated dermal studies with base oils

Material	Duration	Dose (mg/kg)	Effects on skin	Systemic effects	API Report No.
Paraffinic distillates					
Unrefined API 84-01	28 days 3 doses per week	2000	Moderate irritation Proliferative changes	Marginal body weight decrease	33-31642
		1000	Slight iritation	None observed	
		200	Minimal irritation	None observed	
Solvent dewaxed, light API 78-9	21 days 4h/day 3 days/week	5000	Acanthosis, parakeratosis Chronic dermal inflammation	None observed	29-33065
Solvent dewaxed, heavy API 78-10*	"	5000	Acanthosis, parakeratosis Chronic dermal inflammation	None observed	29-33105
79-3	"	5000	None	None observed	29-33067
79-4	"	5000	None	None observed	29-33066
79-5	"	5000	None	None observed	29-33068
5 Paraffinic base oils	28 days 5 days per week	1000	Minor irritation	None observed	Trimmer et al, 1989
Naphthenic distillates					
Solvent refined, light API 78-5	"	5000	Acanthosis, parakeratosis Chronic dermal inflammation	None observed	29-33106
API 79-1	"	5000	None	None observed	29-33065
Hydrotreated, light API 83-12	28 days 3 doses per week	2000	Moderate irritation	Reduced testis weight	33-30499
		1000	Males: slight irritation Females: moderate irritation	None observed	
		200	Minimal irritation	None observed	
Hydrotreated, heavy API 83-15	28 days 3 doses per week	2000	Slight irritation hyperplasia	Elevated SGOT & SGPT, decreased body weight.Subacute hepatitis. Increased relative liver weight in females	35-32430
		1000	Slight irritation	Elevated SGOT & SGPT	
		200	Minimal irritation	None observed	

* Although this material is not included in the HPV Lubricating base stocks category, it is similar to other materials in the category and provides supportive information.